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(54) **Title:** RNA INTERFERENCE MEDIATED INHIBITION OF HEPATITIS C VIRUS (HCV) EXPRESSION USING SHORT INTERFERING NUCLEIC ACID (siNA)

(57) **Abstract:** This invention relates to compounds, compositions, and methods useful for modulating HCVgene expression using short interfering nucleic acid (siNA) molecules. This invention also relates to compounds, compositions, and methods useful for modulating the expression and activity of other genes involved in pathways of HCV gene expression and/or activity by RNA interference (RNAi) using small nucleic acid molecules. In particular, the instant invention features small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules and methods used to modulate the expression of HCV genes.

RNA INTERFERENCE MEDIATED INHIBITION OF HEPATITIS C VIRUS (HCV) EXPRESSION USING SHORT INTERFERING NUCLEIC ACID (siNA)

This invention is a continuation-in-part of U.S. Patent Application No. 10/667,271, filed September 16, 2003, which is a continuation-in-part of International Patent Application No. PCT/US03/05043, filed February 20, 2003, which is a continuation-in-part of McSwiggen PCT/US02/09187, filed March 26, 2002 and claims the benefit of McSwiggen USSN 60/401,104, filed August 5, 2002. This application is also a continuation-in-part of International Patent Application No. PCT/US04/16390, filed May 24, 2004, which is a continuation-in-part of U.S. Patent Application No. 10/826,966, filed April 16, 2004, which is continuation-in-part of U.S. Patent Application No. 10/757,803, filed January 14, 2004, which is a continuation-in-part of U.S. Patent Application No. 10/720,448, filed November 24, 2003, which is a continuation-in-part of U.S. Patent Application No. 10/693,059, filed October 23, 2003, which is a continuation-in-part of U.S. Patent Application No. 10/444,853, filed May 23, 2003, which is a continuation-in-part of International Patent Application No. PCT/US03/05346, filed February 20, 2003, and a continuation-in-part of International Patent Application No. PCT/US03/05028, filed February 20, 2003, both of which claim the benefit of U.S. Provisional Application No. 60/358,580 filed February 20, 2002, U.S. Provisional Application No. 60/363,124 filed March 11, 2002, U.S. Provisional Application No. 60/386,782 filed June 6, 2002, U.S. Provisional Application No. 60/406,784 filed August 29, 2002, U.S. Provisional Application No. 60/408,378 filed September 5, 2002, U.S. Provisional Application No. 60/409,293 filed September 9, 2002, and U.S. Provisional Application No. 60/440,129 filed January 15, 2003. This application is also a continuation-in-part of International Patent Application No. PCT/US04/13456, filed April 30, 2004, which is a continuation-in-part of U.S. Patent Application No. 10/780,447, filed February 13, 2004, which is a continuation-in-part of U.S. Patent Application No. 10/427,160, filed April 30, 2003, which is a continuation-in-part of International Patent Application No. PCT/US02/15876 filed May 17, 2002, which claims the benefit of U.S. Provisional Application No. 60/292,217, filed May 18, 2001, U.S. Provisional Application No. 60/362,016, filed March 6, 2002, U.S. Provisional Application No. 60/306,883, filed July 20, 2001, and U.S. Provisional Application No. 60/311,865, filed August 13, 2001. This application is also a continuation-in-part of U.S. Patent Application No. 10/727,780 filed December 3, 2003. This application also claims

the benefit of U.S. Provisional Application No. 60/543,480, filed February 10, 2004. The instant application claims the benefit of all the listed applications, which are hereby incorporated by reference herein in their entireties, including the drawings.

Field Of The Invention

The present invention relates to compounds, compositions, and methods for the study, diagnosis, and treatment of traits, diseases and conditions that respond to the modulation of hepatitis C virus (HCV) gene expression and/or activity. The present invention is also directed to compounds, compositions, and methods relating to traits, diseases and conditions that respond to the modulation of expression and/or activity of genes involved in hepatitis C virus (HCV) gene expression pathways or other cellular processes that mediate the maintenance or development of such traits, diseases and conditions. Specifically, the invention relates to small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules capable of mediating RNA interference (RNAi) against hepatitis C virus (HCV) gene expression. Such small nucleic acid molecules are useful, for example, in providing compositions to prevent, inhibit, or reduce HCV infection, liver failure, hepatocellular carcinoma, cirrhosis, and/or other disease states associated with HCV infection in a subject or organism.

Background Of The Invention

The following is a discussion of relevant art pertaining to RNAi. The discussion is provided only for understanding of the invention that follows. The summary is not an admission that any of the work described below is prior art to the claimed invention.

RNA interference refers to the process of sequence-specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Zamore *et al.*, 2000, Cell, 101, 25-33; Fire *et al.*, 1998, *Nature*, 391, 806; Hamilton *et al.*, 1999, *Science*, 286, 950-951; Lin *et al.*, 1999, *Nature*, 402, 128-129; Sharp, 1999, Genes & Dev., 13:139-141; and Strauss, 1999, *Science*, 286, 886). The corresponding process in plants (Heifetz *et al.*, International PCT Publication No. WO 99/61631) is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be

an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes and is commonly shared by diverse flora and phyla (Fire *et al.*, 1999, *Trends Genet.*, 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or from the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response through a mechanism that has yet to be fully characterized. This mechanism appears to be different from other known mechanisms involving double stranded RNA-specific ribonucleases, such as the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2',5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L (see for example US Patent Nos. 6,107,094; 5,898,031; Clemens *et al.*, 1997, *J. Interferon & Cytokine Res.*, 17, 503-524; Adah *et al.*, 2001, *Curr. Med. Chem.*, 8, 1189).

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer (Bass, 2000, Cell, 101, 235; Zamore *et al.*, 2000, Cell, 101, 25-33; Hammond *et al.*, 2000, Nature, 404, 293). Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Zamore *et al.*, 2000, Cell, 101, 25-33; Bass, 2000, Cell, 101, 235; Berstein *et al.*, 2001, *Nature*, 409, 363). Short interfering RNAs derived from dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes (Zamore *et al.*, 2000, Cell, 101, 25-33; Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188). Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner *et al.*, 2001, *Science*, 293, 834). The RNAi response also features an endonuclease complex, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence complementary to the antisense strand of the siRNA duplex. Cleavage of the target RNA takes place in the middle of the region complementary to the antisense strand of the siRNA duplex (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188).

RNAi has been studied in a variety of systems. Fire *et al.*, 1998, *Nature*, 391, 806, were the first to observe RNAi in *C. elegans*. Bahramian and Zarbl, 1999, *Molecular and Cellular Biology*, 19, 274-283 and Wianny and Goetz, 1999, *Nature Cell Biol.*, 2,

70, describe RNAi mediated by dsRNA in mammalian systems. Hammond *et al.*, 2000, *Nature,* 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir *et al.*, 2001, *Nature,* 411, 494 and Tuschl *et al.*, International PCT Publication No. WO 01/75164, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in Drosophila embryonic lysates (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877 and Tuschl *et al.*, International PCT Publication No. WO 01/75164) has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21-nucleotide siRNA duplexes are most active when containing 3'-terminal dinucleotide overhangs. Furthermore, complete substitution of one or both siRNA strands with 2'-deoxy (2'-H) or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of the 3'-terminal siRNA overhang nucleotides with 2'-deoxy nucleotides (2'-H) was shown to be tolerated. Single mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end of the guide sequence (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen *et al.*, 2001, *Cell,* 107, 309).

Studies have shown that replacing the 3'-terminal nucleotide overhanging segments of a 21-mer siRNA duplex having two-nucleotide 3'-overhangs with deoxyribonucleotides does not have an adverse effect on RNAi activity. Replacing up to four nucleotides on each end of the siRNA with deoxyribonucleotides has been reported to be well tolerated, whereas complete substitution with deoxyribonucleotides results in no RNAi activity (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877 and Tuschl *et al.*, International PCT Publication No. WO 01/75164). In addition, Elbashir *et al., supra*, also report that substitution of siRNA with 2'-O-methyl nucleotides completely abolishes RNAi activity. Li *et al.*, International PCT Publication No. WO 00/44914, and Beach *et al.*, International PCT Publication No. WO 01/68836 preliminarily suggest that siRNA may include modifications to either the phosphate-sugar backbone or the nucleoside to include at least one of a nitrogen or sulfur heteroatom, however, neither application postulates to what extent such modifications would be tolerated in siRNA molecules, nor

provides any further guidance or examples of such modified siRNA. Kreutzer *et al.*, Canadian Patent Application No. 2,359,180, also describe certain chemical modifications for use in dsRNA constructs in order to counteract activation of double-stranded RNA-dependent protein kinase PKR, specifically 2'-amino or 2'-O-methyl nucleotides, and nucleotides containing a 2'-O or 4'-C methylene bridge. However, Kreutzer *et al.* similarly fails to provide examples or guidance as to what extent these modifications would be tolerated in dsRNA molecules.

Parrish *et al.*, 2000, *Molecular Cell*, 6, 1077-1087, tested certain chemical modifications targeting the unc-22 gene in *C. elegans* using long (>25 nt) siRNA transcripts. The authors describe the introduction of thiophosphate residues into these siRNA transcripts by incorporating thiophosphate nucleotide analogs with T7 and T3 RNA polymerase and observed that RNAs with two phosphorothioate modified bases also had substantial decreases in effectiveness as RNAi. Further, Parrish *et al.* reported that phosphorothioate modification of more than two residues greatly destabilized the RNAs *in vitro* such that interference activities could not be assayed. *Id.* at 1081. The authors also tested certain modifications at the 2'-position of the nucleotide sugar in the long siRNA transcripts and found that substituting deoxynucleotides for ribonucleotides produced a substantial decrease in interference activity, especially in the case of Uridine to Thymidine and/or Cytidine to deoxy-Cytidine substitutions. *Id.* In addition, the authors tested certain base modifications, including substituting, in sense and antisense strands of the siRNA, 4-thiouracil, 5-bromouracil, 5-iodouracil, and 3-(aminoallyl)uracil for uracil, and inosine for guanosine. Whereas 4-thiouracil and 5-bromouracil substitution appeared to be tolerated, Parrish reported that inosine produced a substantial decrease in interference activity when incorporated in either strand. Parrish also reported that incorporation of 5-iodouracil and 3-(aminoallyl)uracil in the antisense strand resulted in a substantial decrease in RNAi activity as well.

The use of longer dsRNA has been described. For example, Beach *et al.*, International PCT Publication No. WO 01/68836, describes specific methods for attenuating gene expression using endogenously-derived dsRNA. Tuschl *et al.*, International PCT Publication No. WO 01/75164, describe a *Drosophila in vitro* RNAi system and the use of specific siRNA molecules for certain functional genomic and certain therapeutic applications; although Tuschl, 2001, *Chem. Biochem.*, 2, 239-245, doubts that RNAi can be used to cure genetic diseases or viral infection due to the danger

of activating interferon response. Li *et al.*, International PCT Publication No. WO 00/44914, describe the use of specific long (141 bp-488 bp) enzymatically synthesized or vector expressed dsRNAs for attenuating the expression of certain target genes. Zernicka-Goetz *et al.*, International PCT Publication No. WO 01/36646, describe certain methods for inhibiting the expression of particular genes in mammalian cells using certain long (550 bp-714 bp), enzymatically synthesized or vector expressed dsRNA molecules. Fire *et al.*, International PCT Publication No. WO 99/32619, describe particular methods for introducing certain long dsRNA molecules into cells for use in inhibiting gene expression in nematodes. Plaetinck *et al.*, International PCT Publication No. WO 00/01846, describe certain methods for identifying specific genes responsible for conferring a particular phenotype in a cell using specific long dsRNA molecules. Mello *et al.*, International PCT Publication No. WO 01/29058, describe the identification of specific genes involved in dsRNA-mediated RNAi. Pachuck *et al.*, International PCT Publication No. WO 00/63364, describe certain long (at least 200 nucleotide) dsRNA constructs. Deschamps Depaillette *et al.*, International PCT Publication No. WO 99/07409, describe specific compositions consisting of particular dsRNA molecules combined with certain anti-viral agents. Waterhouse *et al.*, International PCT Publication No. 99/53050 and 1998, *PNAS*, 95, 13959-13964, describe certain methods for decreasing the phenotypic expression of a nucleic acid in plant cells using certain dsRNAs. Driscoll *et al.*, International PCT Publication No. WO 01/49844, describe specific DNA expression constructs for use in facilitating gene silencing in targeted organisms.

Others have reported on various RNAi and gene-silencing systems. For example, Parrish *et al.*, 2000, *Molecular Cell*, 6, 1077-1087, describe specific chemically-modified dsRNA constructs targeting the unc-22 gene of *C. elegans*. Grossniklaus, International PCT Publication No. WO 01/38551, describes certain methods for regulating polycomb gene expression in plants using certain dsRNAs. Churikov *et al.*, International PCT Publication No. WO 01/42443, describe certain methods for modifying genetic characteristics of an organism using certain dsRNAs. Cogoni *et al,*, International PCT Publication No. WO 01/53475, describe certain methods for isolating a Neurospora silencing gene and uses thereof. Reed *et al.*, International PCT Publication No. WO 01/68836, describe certain methods for gene silencing in plants. Honer *et al.*, International PCT Publication No. WO 01/70944, describe certain methods of drug

screening using transgenic nematodes as Parkinson's Disease models using certain dsRNAs. Deak *et al.*, International PCT Publication No. WO 01/72774, describe certain *Drosophila*-derived gene products that may be related to RNAi in *Drosophila*. Arndt *et al.*, International PCT Publication No. WO 01/92513 describe certain methods for mediating gene suppression by using factors that enhance RNAi. Tuschl *et al.*, International PCT Publication No. WO 02/44321, describe certain synthetic siRNA constructs. Pachuk *et al.*, International PCT Publication No. WO 00/63364, and Satishchandran *et al.*, International PCT Publication No. WO 01/04313, describe certain methods and compositions for inhibiting the function of certain polynucleotide sequences using certain long (over 250 bp), vector expressed dsRNAs. Echeverri *et al.*, International PCT Publication No. WO 02/38805, describe certain *C. elegans* genes identified via RNAi. Kreutzer *et al.*, International PCT Publications Nos. WO 02/055692, WO 02/055693, and EP 1144623 B1 describes certain methods for inhibiting gene expression using dsRNA. Graham *et al.*, International PCT Publications Nos. WO 99/49029 and WO 01/70949, and AU 4037501 describe certain vector expressed siRNA molecules. Fire *et al.*, US 6,506,559, describe certain methods for inhibiting gene expression in vitro using certain long dsRNA (299 bp-1033 bp) constructs that mediate RNAi. Martinez *et al.*, 2002, *Cell*, 110, 563-574, describe certain single stranded siRNA constructs, including certain 5'-phosphorylated single stranded siRNAs that mediate RNA interference in Hela cells. Harborth *et al.*, 2003, Antisense & Nucleic Acid Drug Development, 13, 83-105, describe certain chemically and structurally modified siRNA molecules. Chiu and Rana, 2003, RNA, 9, 1034-1048, describe certain chemically and structurally modified siRNA molecules. Woolf *et al.*, International PCT Publication Nos. WO 03/064626 and WO 03/064625 describe certain chemically modified dsRNA constructs.

McCaffrey *et al.*, 2002, *Nature*, 418, 38-39, describes the use of certain siRNA constructs targeting a chimeric HCV NS5B protein/luciferase transcript in mice.

Randall *et al.*, 2003, *PNAS USA*, 100, 235-240, describe certain siRNA constructs targeting HCV RNA in Huh7 hepatoma cell lines.

SUMMARY OF THE INVENTION

This invention relates to compounds, compositions, and methods useful for modulating the expression of genes, such as those genes associated with the development

or maintenance of HCV infection, liver failure, hepatocellular carcinoma, cirrhosis, and/or other disease states associated with HCV infection, by RNA interference (RNAi) using short interfering nucleic acid (siNA) molecules. This invention also relates to compounds, compositions, and methods useful for modulating the expression and activity of other genes involved in pathways of HCV gene expression and/or activity by RNA interference (RNAi) using small nucleic acid molecules. In particular, the instant invention features small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules and methods used to modulate the expression of HCV genes and/or other genes (e.g., cellular or host genes) involved in pathways of HCV gene expression and/or infection.

A siNA of the invention can be unmodified or chemically-modified. A siNA of the instant invention can be chemically synthesized, expressed from a vector or enzymatically synthesized. The instant invention also features various chemically-modified synthetic short interfering nucleic acid (siNA) molecules capable of modulating HCV gene expression or activity in cells by RNA interference (RNAi). The use of chemically-modified siNA improves various properties of native siNA molecules through increased resistance to nuclease degradation *in vivo* and/or through improved cellular uptake. Further, contrary to earlier published studies, siNA having multiple chemical modifications retains its RNAi activity. The siNA molecules of the instant invention provide useful reagents and methods for a variety of therapeutic, veterinary, diagnostic, target validation, genomic discovery, genetic engineering, and pharmacogenomic applications.

In one embodiment, the invention features one or more siNA molecules and methods that independently or in combination modulate the expression of gene(s) encoding HCV and/or cellular proteins associated with the maintenance or development of HCV infection, liver failure, hepatocellular carcinoma, and cirrhosis, such as genes encoding sequences comprising those sequences referred to by GenBank Accession Nos. shown in **Table I**, referred to herein generally as HCV. The description below of the various aspects and embodiments of the invention is provided with reference to exemplary hepatitis C virus (HCV) genes, generally referred to herein as HCV. However, such reference is meant to be exemplary only and the various aspects and embodiments of the invention are also directed to other genes that express alternate HCV

genes, such as mutant HCV genes, splice variants of HCV genes, and genes encoding different strains of HCV, as well as as cellular targets for HCV, such as those described herein. The various aspects and embodiments are also directed to other genes involved in HCV pathways, including genes that encode cellular proteins involved in the maintenance and/or development of HCV infection, liver failure, hepatocellular carcinoma, and cirrhosis or other genes that express other proteins associated with HCV infection, such as cellular proteins that are utilized in the HCV life-cycle. Such additional genes can be analyzed for target sites using the methods described herein for HCV. Thus, the inhibition and the effects of such inhibition of the other genes can be performed as described herein. In other words, the term "HCV" as it is defined herein below and recited in the described embodiments, is meant to encompass genes associated with the development and/or maintenance of HCV infection, such as genes which encode HCV polypeptides, including polypeptides of different strains of HCV, mutant HCV genes, and splice variants of HCV genes, as well as cellular genes involved in HCV pathways of gene expression, replication, and/or HCV activity. Also, the term "HCV" as it is defined herein below and recited in the described embodiments, is meant to encompass HCV viral gene products and cellular gene products involved in HCV infection, such as those described herein. Thus, each of the embodiments described herein with reference to the term "HCV" are applicable to all of the virus, cellular and viral protein, peptide, polypeptide, and/or polynucleotide molecules covered by the term "HCV", as that term is defined herein.

In one embodiment, the invention features siNA molecules having RNAi specificity for the HCV minus strand, for example, Genbank Accession No. HPCK1S1, Hepatitis C virus (strain HCV-1b, clone HCV-K1-S1), complete genome; Genbank Accession No. D50483, 9410 nt.

In one embodiment, the invention features one or more siNA molecules and methods that independently or in combination modulate the expression of genes representing cellular targets for HCV infection, such as cellular receptors, cell surface molecules, cellular enzymes, cellular transcription factors, and/or cytokines, second messengers, and cellular accessory molecules including, but not limited to, La antigen (see for example Costa-Mattioli *et al.*, 2004, *Mol Cell Biol.*, 24, 6861-70, e.g., Genbank Accession No. NM_003142); FAS (e.g., Genbank Accession No. NM_000043) or FAS ligand (e.g., Genbank Accession No. NM_000639); interferon regulatory factors (IRFs;

e.g., Genbank Accession No. AF082503.1); cellular PKR protein kinase (e.g., Genbank Accession No. XM_002661.7); human eukaryotic initiation factors 2B (elF2Bgamma; e.g., Genbank Accession No. AF256223, and/or elF2gamma; e.g., Genbank Accession No. NM_006874.1); human DEAD Box protein (DDX3; e.g., Genbank Accession No. XM_018021.2); and cellular proteins that bind to the poly(U) tract of the HCV 3'-UTR, such as polypyrimidine tract-binding protein (e.g., Genbank Accession Nos. NM_031991.1 and XM_042972.3). Such cellular targets are also referred to herein generally as HCV targets, and specifically as "host target" or "host targets".

Due to the high sequence variability of the HCV genome, selection of siNA molecules for broad therapeutic applications likely involve the conserved regions of the HCV genome. In one embodiment, the present invention relates to siNA molecules that target the conserved regions of the HCV genome. Examples of conserved regions of the HCV genome include, but are not limited to, the 5'-Non Coding Region (NCR, also referred to as the 5'-untranslated region, UTR), the 5'-end of the core protein coding region, and the 3'- NCR. HCV genomic RNA contains an internal ribosome entry site (IRES) in the 5'-NCR which mediates translation independently of a 5'-cap structure (Wang *et al.*, 1993, *J. Virol.*, 67, 3338-44). The full-length sequence of the HCV RNA genome is heterologous among clinically isolated subtypes, of which there are at least fifteen (Simmonds, 1995, *Hepatology*, 21, 570-583), however, the 5'-NCR sequence of HCV is highly conserved across all known subtypes, most likely to preserve the shared IRES mechanism (Okamoto *et al.*, 1991, *J. General Virol.*, 72, 2697-2704). Therefore, a siNA molecule can be designed to target the different isolates of HCV by targeting a conserved region, such as the 5' NCR sequence. siNA molecules designed to target conserved regions of various HCV isolates enable efficient inhibition of HCV replication in diverse patient populations and ensure the effectiveness of the siNA molecules against HCV quasi species which evolve due to mutations in the non-conserved regions of the HCV genome. As described, a single siNA molecule can be targeted against all isolates of HCV by designing the siNA molecule to interact with conserved nucleotide sequences of HCV (e.g., sequences that are expected to be present in the RNA of various HCV isolates).

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a HCV gene or that

directs cleavage of a HCV RNA, wherein said siNA molecule comprises about 15 to about 28 base pairs.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a HCV RNA via RNA interference (RNAi), wherein the double stranded siNA molecule comprises a first and a second strand, each strand of the siNA molecule is about 18 to about 28 nucleotides in length, the first strand of the siNA molecule comprises nucleotide sequence having sufficient complementarity to the HCV RNA for the siNA molecule to direct cleavage of the HCV RNA via RNA interference, and the second strand of said siNA molecule comprises nucleotide sequence that is complementary to the first strand.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a HCV RNA via RNA interference (RNAi), wherein the double stranded siNA molecule comprises a first and a second strand, each strand of the siNA molecule is about 18 to about 23 nucleotides in length, the first strand of the siNA molecule comprises nucleotide sequence having sufficient complementarity to the HCV RNA for the siNA molecule to direct cleavage of the HCV RNA via RNA interference, and the second strand of said siNA molecule comprises nucleotide sequence that is complementary to the first strand.

In one embodiment, the invention features a chemically synthesized double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a HCV RNA via RNA interference (RNAi), wherein each strand of the siNA molecule is about 18 to about 28 nucleotides in length; and one strand of the siNA molecule comprises nucleotide sequence having sufficient complementarity to the HCV RNA for the siNA molecule to direct cleavage of the HCV RNA via RNA interference.

In one embodiment, the invention features a chemically synthesized double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a HCV RNA via RNA interference (RNAi), wherein each strand of the siNA molecule is about 18 to about 23 nucleotides in length; and one strand of the siNA molecule comprises nucleotide sequence having sufficient complementarity to the HCV RNA for the siNA molecule to direct cleavage of the HCV RNA via RNA interference.

In one embodiment, the invention features a siNA molecule that down-regulates expression of a HCV gene or that directs cleavage of a HCV RNA, for example, wherein the HCV gene or RNA comprises HCV encoding sequence. In one embodiment, the invention features a siNA molecule that down-regulates expression of a HCV gene or that directs cleavage of a HCV RNA, for example, wherein the HCV gene of RNA comprises HCV non-coding sequence or regulatory elements involved in HCV gene expression.

In one embodiment, a siNA of the invention is used to inhibit the expression of HCV genes or a HCV gene family (e.g., different HCV strains), wherein the genes or gene family sequences share sequence homology. Such homologous sequences can be identified as is known in the art, for example using sequence alignments. siNA molecules can be designed to target such homologous sequences, for example using perfectly complementary sequences or by incorporating non-canonical base pairs, for example mismatches and/or wobble base pairs, that can provide additional target sequences. In instances where mismatches are identified, non-canonical base pairs (for example, mismatches and/or wobble bases) can be used to generate siNA molecules that target more than one gene sequence. In a non-limiting example, non-canonical base pairs such as UU and CC base pairs are used to generate siNA molecules that are capable of targeting sequences for differing HCV targets that share sequence homology. As such, one advantage of using siNAs of the invention is that a single siNA can be designed to include nucleic acid sequence that is complementary to the nucleotide sequence that is conserved between the homologous genes. In this approach, a single siNA can be used to inhibit expression of more than one gene instead of using more than one siNA molecule to target the different genes.

In one embodiment, the invention features a siNA molecule having RNAi activity against HCV RNA, wherein the siNA molecule comprises a sequence complementary to any RNA having HCV encoding sequence, such as those sequences having GenBank Accession Nos. shown in **Table I**. In another embodiment, the invention features a siNA molecule having RNAi activity against HCV RNA, wherein the siNA molecule comprises a sequence complementary to an RNA having variant HCV encoding sequence, for example other mutant HCV genes not shown in **Table I** but known in the art to be associated with, for example, the maintenance and/or development of HCV infection, liver failure, hepatocellular carcinoma, or cirrhosis. Chemical modifications as

shown in **Tables III and IV** or otherwise described herein can be applied to any siNA construct of the invention. In another embodiment, a siNA molecule of the invention includes a nucleotide sequence that can interact with nucleotide sequence of a HCV gene and thereby mediate silencing of HCV gene expression, for example, wherein the siNA mediates regulation of HCV gene expression by cellular processes that modulate the transcription or translation of the HCV gene and prevent expression of the HCV gene.

In one embodiment, siNA molecules of the invention are used to down regulate or inhibit the expression of proteins arising from haplotype polymorphisms (e.g., cellular genes involved in HCV infection or replication) that are associated with a trait, disease or condition. Analysis of genes, or protein or RNA levels can be used to identify subjects with such polymorphisms or those subjects who are at risk of developing traits, conditions, or diseases described herein (see for example Silvestri *et al.*, 2003, *Int J Cancer.*, 104, 310-7). These subjects are amenable to treatment, for example, treatment with siNA molecules of the invention and any other composition useful in treating diseases related to HCV gene expression. As such, analysis of HCV protein or RNA levels can be used to determine treatment type and the course of therapy in treating a subject. Monitoring of HCV protein or RNA levels can be used to predict treatment outcome and to determine the efficacy of compounds and compositions that modulate the level and/or activity of certain HCV proteins associated with a trait, condition, or disease.

In one embodiment of the invention a siNA molecule comprises an antisense strand comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof encoding a HCV protein. The siNA further comprises a sense strand, wherein said sense strand comprises a nucleotide sequence of a HCV gene or a portion thereof.

In another embodiment, a siNA molecule comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence encoding a HCV protein or a portion thereof. The siNA molecule further comprises a sense region, wherein said sense region comprises a nucleotide sequence of a HCV gene or a portion thereof.

In another embodiment, the invention features a siNA molecule comprising a nucleotide sequence in the antisense region of the siNA molecule that is complementary

to a nucleotide sequence or portion of sequence of a HCV gene. In another embodiment, the invention features a siNA molecule comprising a region, for example, the antisense region of the siNA construct, complementary to a sequence comprising a HCV gene sequence or a portion thereof.

In one embodiment, the antisense region of HCV siNA constructs comprises a sequence complementary to sequence having any of SEQ ID NOs. 1-696 or 1393-1466. In one embodiment, the antisense region of HCV constructs comprises sequence having any of antisense SEQ ID NOs. in Tables II and III and Figures 4 and 5. In another embodiment, the sense region of HCV constructs comprises sequence having any of sense SEQ ID NOs. in Tables II and III and Figures 4 and 5.

In one embodiment, a siNA molecule of the invention comprises any of SEQ ID NOs. 1-2027. The sequences shown in SEQ ID NOs: 1-2027 are not limiting. A siNA molecule of the invention can comprise any contiguous HCV sequence (e.g., about 15 to about 25 or more, or about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 or more contiguous HCV nucleotides).

In yet another embodiment, the invention features a siNA molecule comprising a sequence, for example, the antisense sequence of the siNA construct, complementary to a sequence or portion of sequence comprising sequence represented by GenBank Accession Nos. shown in **Table I**. Chemical modifications in **Tables III and IV** and described herein can be applied to any siNA construct of the invention.

In one embodiment of the invention a siNA molecule comprises an antisense strand having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein the antisense strand is complementary to a RNA sequence or a portion thereof encoding HCV or a HCV protein, and wherein said siNA further comprises a sense strand having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, and wherein said sense strand and said antisense strand are distinct nucleotide sequences where at least about 15 nucleotides in each strand are complementary to the other strand.

In another embodiment of the invention a siNA molecule of the invention comprises an antisense region having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein the antisense region is

complementary to a RNA sequence encoding HCV or a HCV protein, and wherein said siNA further comprises a sense region having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein said sense region and said antisense region are comprised in a linear molecule where the sense region comprises at least about 15 nucleotides that are complementary to the antisense region.

In one embodiment, a siNA molecule of the invention has RNAi activity that modulates expression of RNA encoded by a HCV gene. Because HCV genes can share some degree of sequence homology with each other, siNA molecules can be designed to target a class of HCV genes (e.g., a class of different HCV strains) or alternately specific HCV genes (e.g., escape mutants, resistant strains, or other polymorphic variants) by selecting sequences that are either shared amongst different HCV targets or alternatively that are unique for a specific HCV target. Therefore, in one embodiment, the siNA molecule can be designed to target conserved regions of HCV RNA sequences having homology among several HCV gene variants so as to target a class of HCV genes with one siNA molecule. Accordingly, in one embodiment, the siNA molecule of the invention modulates the expression of one or more HCV stains in a subject or organism. In another embodiment, the siNA molecule can be designed to target a sequence that is unique to a specific HCV RNA sequence (e.g., a single HCV strain or HCV single nucleotide polymorphism (SNP)) due to the high degree of specificity that the siNA molecule requires to mediate RNAi activity.

In one embodiment, nucleic acid molecules of the invention that act as mediators of the RNA interference gene silencing response are double-stranded nucleic acid molecules. In another embodiment, the siNA molecules of the invention consist of duplex nucleic acid molecules containing about 15 to about 30 base pairs between oligonucleotides comprising about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides. In yet another embodiment, siNA molecules of the invention comprise duplex nucleic acid molecules with overhanging ends of about 1 to about 3 (*e.g.*, about 1, 2, or 3) nucleotides, for example, about 21-nucleotide duplexes with about 19 base pairs and 3'-terminal mononucleotide, dinucleotide, or trinucleotide overhangs. In yet another embodiment, siNA molecules of the invention comprise duplex nucleic acid molecules with blunt ends, where both ends are blunt, or alternatively, where one of the ends is blunt.

In one embodiment, the invention features one or more chemically-modified siNA constructs having specificity for HCV expressing nucleic acid molecules, such as RNA encoding a HCV protein. In one embodiment, the invention features a RNA based siNA molecule (e.g., a siNA comprising 2'-OH nucleotides) having specificity for HCV expressing nucleic acid molecules that includes one or more chemical modifications described herein. Non-limiting examples of such chemical modifications include without limitation phosphorothioate internucleotide linkages, 2'-deoxyribonucleotides, 2'-O-methyl ribonucleotides, 2'-deoxy-2'-fluoro ribonucleotides, "universal base" nucleotides, "acyclic" nucleotides, 5-C-methyl nucleotides, and terminal glyceryl and/or inverted deoxy abasic residue incorporation. These chemical modifications, when used in various siNA constructs, (e.g., RNA based siNA constructs), are shown to preserve RNAi activity in cells while at the same time, dramatically increasing the serum stability of these compounds. Furthermore, contrary to the data published by Parrish *et al., supra,* applicant demonstrates that multiple (greater than one) phosphorothioate substitutions are well-tolerated and confer substantial increases in serum stability for modified siNA constructs.

In one embodiment, a siNA molecule of the invention comprises modified nucleotides while maintaining the ability to mediate RNAi. The modified nucleotides can be used to improve *in vitro* or *in vivo* characteristics such as stability, activity, and/or bioavailability. For example, a siNA molecule of the invention can comprise modified nucleotides as a percentage of the total number of nucleotides present in the siNA molecule. As such, a siNA molecule of the invention can generally comprise about 5% to about 100% modified nucleotides (e.g., about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% modified nucleotides). The actual percentage of modified nucleotides present in a given siNA molecule will depend on the total number of nucleotides present in the siNA. If the siNA molecule is single stranded, the percent modification can be based upon the total number of nucleotides present in the single stranded siNA molecules. Likewise, if the siNA molecule is double stranded, the percent modification can be based upon the total number of nucleotides present in the sense strand, antisense strand, or both the sense and antisense strands.

One aspect of the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a HCV gene or that directs

cleavage of a HCV RNA. In one embodiment, the double stranded siNA molecule comprises one or more chemical modifications and each strand of the double-stranded siNA is about 21 nucleotides long. In one embodiment, the double-stranded siNA molecule does not contain any ribonucleotides. In another embodiment, the double-stranded siNA molecule comprises one or more ribonucleotides. In one embodiment, each strand of the double-stranded siNA molecule independently comprises about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein each strand comprises about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to the nucleotides of the other strand. In one embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of the HCV gene, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence of the HCV gene or a portion thereof.

In another embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a HCV gene or that directs cleavage of a HCV RNA, comprising an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of the HCV gene or a portion thereof, and a sense region, wherein the sense region comprises a nucleotide sequence substantially similar to the nucleotide sequence of the HCV gene or a portion thereof. In one embodiment, the antisense region and the sense region independently comprise about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein the antisense region comprises about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to nucleotides of the sense region.

In another embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a HCV gene or that directs cleavage of a HCV RNA, comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the HCV gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region.

In one embodiment, a siNA molecule of the invention comprises blunt ends, i.e., ends that do not include any overhanging nucleotides. For example, a siNA molecule comprising modifications described herein (e.g., comprising nucleotides having Formulae I-VII or siNA constructs comprising "Stab 00"-"Stab 32" (**Table IV**) or any combination thereof (see **Table IV**)) and/or any length described herein can comprise blunt ends or ends with no overhanging nucleotides.

In one embodiment, any siNA molecule of the invention can comprise one or more blunt ends, i.e. where a blunt end does not have any overhanging nucleotides. In one embodiment, the blunt ended siNA molecule has a number of base pairs equal to the number of nucleotides present in each strand of the siNA molecule. In another embodiment, the siNA molecule comprises one blunt end, for example wherein the 5'-end of the antisense strand and the 3'-end of the sense strand do not have any overhanging nucleotides. In another example, the siNA molecule comprises one blunt end, for example wherein the 3'-end of the antisense strand and the 5'-end of the sense strand do not have any overhanging nucleotides. In another example, a siNA molecule comprises two blunt ends, for example wherein the 3'-end of the antisense strand and the 5'-end of the sense strand as well as the 5'-end of the antisense strand and 3'-end of the sense strand do not have any overhanging nucleotides. A blunt ended siNA molecule can comprise, for example, from about 15 to about 30 nucleotides (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides). Other nucleotides present in a blunt ended siNA molecule can comprise, for example, mismatches, bulges, loops, or wobble base pairs to modulate the activity of the siNA molecule to mediate RNA interference.

By "blunt ends" is meant symmetric termini or termini of a double stranded siNA molecule having no overhanging nucleotides. The two strands of a double stranded siNA molecule align with each other without over-hanging nucleotides at the termini. For example, a blunt ended siNA construct comprises terminal nucleotides that are complementary between the sense and antisense regions of the siNA molecule.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a HCV gene or that directs cleavage of a HCV RNA, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and

the second fragment comprises the antisense region of the siNA molecule. The sense region can be connected to the antisense region via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker.

In one embodiment, the invention features double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a HCV gene or that directs cleavage of a HCV RNA, wherein the siNA molecule comprises about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) base pairs, and wherein each strand of the siNA molecule comprises one or more chemical modifications. In another embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence of a HCV gene or a portion thereof, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of the HCV gene. In another embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence of a HCV gene or portion thereof, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or portion thereof of the HCV gene. In another embodiment, each strand of the siNA molecule comprises about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, and each strand comprises at least about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to the nucleotides of the other strand. The HCV gene can comprise, for example, sequences referred to in **Table I**.

In one embodiment, a siNA molecule of the invention comprises no ribonucleotides. In another embodiment, a siNA molecule of the invention comprises ribonucleotides.

In one embodiment, a siNA molecule of the invention comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence of a HCV gene or a portion thereof, and the siNA further comprises a sense region comprising a nucleotide sequence substantially similar to the nucleotide sequence of the HCV gene or a portion thereof. In another embodiment, the antisense region and the sense region each comprise about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21,

22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides and the antisense region comprises at least about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to nucleotides of the sense region. The HCV gene can comprise, for example, sequences referred to in **Table I**. In another embodiment, the siNA is a double stranded nucleic acid molecule, where each of the two strands of the siNA molecule independently comprise about 15 to about 40 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 23, 33, 34, 35, 36, 37, 38, 39, or 40) nucleotides, and where one of the strands of the siNA molecule comprises at least about 15 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 or more) nucleotides that are complementary to the nucleic acid sequence of the HCV gene or a portion thereof.

In one embodiment, a siNA molecule of the invention comprises a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by a HCV gene, or a portion thereof, and the sense region comprises a nucleotide sequence that is complementary to the antisense region. In one embodiment, the siNA molecule is assembled from two separate oligonucleotide fragments, wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule. In another embodiment, the sense region is connected to the antisense region via a linker molecule. In another embodiment, the sense region is connected to the antisense region via a linker molecule, such as a nucleotide or non-nucleotide linker. The HCV gene can comprise, for example, sequences referred in to **Table I**.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a HCV gene or that directs cleavage of a HCV RNA, comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the HCV gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region, and wherein the siNA molecule has one or more modified pyrimidine and/or purine nucleotides. In one embodiment, the pyrimidine nucleotides in the sense region are 2'-O-methyl pyrimidine nucleotides or 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In

another embodiment, the pyrimidine nucleotides in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides. In another embodiment, the pyrimidine nucleotides in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In one embodiment, the pyrimidine nucleotides in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the antisense region are 2'-O-methyl or 2'-deoxy purine nucleotides. In another embodiment of any of the above-described siNA molecules, any nucleotides present in a non-complementary region of the sense strand (e.g. overhang region) are 2'-deoxy nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a HCV gene or that directs cleavage of a HCV RNA, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule, and wherein the fragment comprising the sense region includes a terminal cap moiety at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the fragment. In one embodiment, the terminal cap moiety is an inverted deoxy abasic moiety or glyceryl moiety. In one embodiment, each of the two fragments of the siNA molecule independently comprise about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides. In another embodiment, each of the two fragments of the siNA molecule independently comprise about 15 to about 40 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 23, 33, 34, 35, 36, 37, 38, 39, or 40) nucleotides. In a non-limiting example, each of the two fragments of the siNA molecule comprise about 21 nucleotides.

In one embodiment, the invention features a siNA molecule comprising at least one modified nucleotide, wherein the modified nucleotide is a 2'-deoxy-2'-fluoro nucleotide. The siNA can be, for example, about 15 to about 40 nucleotides in length. In one embodiment, all pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In one embodiment, the modified nucleotides in the siNA include at least one 2'-deoxy-2'-fluoro cytidine or 2'-deoxy-2'-fluoro uridine nucleotide. In another embodiment, the modified nucleotides in the siNA include at least one 2'-fluoro cytidine and at least one 2'-deoxy-2'-fluoro uridine nucleotides. In one

embodiment, all uridine nucleotides present in the siNA are 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all cytidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro cytidine nucleotides. In one embodiment, all adenosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro adenosine nucleotides. In one embodiment, all guanosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro guanosine nucleotides. The siNA can further comprise at least one modified internucleotidic linkage, such as phosphorothioate linkage. In one embodiment, the 2'-deoxy-2'-fluoronucleotides are present at specifically selected locations in the siNA that are sensitive to cleavage by ribonucleases, such as locations having pyrimidine nucleotides.

In one embodiment, the invention features a method of increasing the stability of a siNA molecule against cleavage by ribonucleases comprising introducing at least one modified nucleotide into the siNA molecule, wherein the modified nucleotide is a 2'-deoxy-2'-fluoro nucleotide. In one embodiment, all pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In one embodiment, the modified nucleotides in the siNA include at least one 2'-deoxy-2'-fluoro cytidine or 2'-deoxy-2'-fluoro uridine nucleotide. In another embodiment, the modified nucleotides in the siNA include at least one 2'-fluoro cytidine and at least one 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all uridine nucleotides present in the siNA are 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all cytidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro cytidine nucleotides. In one embodiment, all adenosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro adenosine nucleotides. In one embodiment, all guanosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro guanosine nucleotides. The siNA can further comprise at least one modified internucleotidic linkage, such as phosphorothioate linkage. In one embodiment, the 2'-deoxy-2'-fluoronucleotides are present at specifically selected locations in the siNA that are sensitive to cleavage by ribonucleases, such as locations having pyrimidine nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a HCV gene or that directs cleavage of a HCV RNA, comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the HCV gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense

region, and wherein the purine nucleotides present in the antisense region comprise 2'-deoxy- purine nucleotides. In an alternative embodiment, the purine nucleotides present in the antisense region comprise 2'-O-methyl purine nucleotides. In either of the above embodiments, the antisense region can comprise a phosphorothioate internucleotide linkage at the 3' end of the antisense region. Alternatively, in either of the above embodiments, the antisense region can comprise a glyceryl modification at the 3' end of the antisense region. In another embodiment of any of the above-described siNA molecules, any nucleotides present in a non-complementary region of the antisense strand (e.g. overhang region) are 2'-deoxy nucleotides.

In one embodiment, the antisense region of a siNA molecule of the invention comprises sequence complementary to a portion of an endogenous transcript having sequence unique to a particular HCV disease related allele in a subject or organism, such as sequence comprising a single nucleotide polymorphism (SNP) associated with the disease specific allele. As such, the antisense region of a siNA molecule of the invention can comprise sequence complementary to sequences that are unique to a particular allele to provide specificity in mediating selective RNAi against the disease, condition, or trait related allele.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a HCV gene or that directs cleavage of a HCV RNA, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule, where each strand is about 21 nucleotides long and where about 19 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule, wherein at least two 3' terminal nucleotides of each fragment of the siNA molecule are not base-paired to the nucleotides of the other fragment of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule, where each strand is about 19 nucleotide long and where the nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule to form at least about 15 (e.g., 15, 16, 17, 18, or 19) base pairs, wherein one or both ends of the siNA molecule are blunt ends. In one embodiment, each of the two 3' terminal nucleotides of

each fragment of the siNA molecule is a 2'-deoxy-pyrimidine nucleotide, such as a 2'-deoxy-thymidine. In another embodiment, all nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule of about 19 to about 25 base pairs having a sense region and an antisense region, where about 19 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the HCV gene. In another embodiment, about 21 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the HCV gene. In any of the above embodiments, the 5'-end of the fragment comprising said antisense region can optionally include a phosphate group.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a HCV RNA sequence (e.g., wherein said target RNA sequence is encoded by a HCV gene involved in the HCV pathway), wherein the siNA molecule does not contain any ribonucleotides and wherein each strand of the double-stranded siNA molecule is about 15 to about 30 nucleotides. In one embodiment, the siNA molecule is 21 nucleotides in length. Examples of non-ribonucleotide containing siNA constructs are combinations of stabilization chemistries shown in **Table IV** in any combination of Sense/Antisense chemistries, such as Stab 7/8, Stab 7/11, Stab 8/8, Stab 18/8, Stab 18/11, Stab 12/13, Stab 7/13, Stab 18/13, Stab 7/19, Stab 8/19, Stab 18/19, Stab 7/20, Stab 8/20, Stab 18/20, Stab 7/32, Stab 8/32, or Stab 18/32 (e.g., any siNA having Stab 7, 8, 11, 12, 13, 14, 15, 17, 18, 19, 20, or 32 sense or antisense strands or any combination thereof).

In one embodiment, the invention features a chemically synthesized double stranded RNA molecule that directs cleavage of a HCV RNA via RNA interference, wherein each strand of said RNA molecule is about 15 to about 30 nucleotides in length; one strand of the RNA molecule comprises nucleotide sequence having sufficient complementarity to the HCV RNA for the RNA molecule to direct cleavage of the HCV RNA via RNA interference; and wherein at least one strand of the RNA molecule optionally comprises one or more chemically modified nucleotides described herein, such as without limitation deoxynucleotides, 2'-O-methyl nucleotides, 2'-deoxy-2'-fluoro nucleotides, 2'-O-methoxyethyl nucleotides etc.

In one embodiment, the invention features a medicament comprising a siNA molecule of the invention.

In one embodiment, the invention features an active ingredient comprising a siNA molecule of the invention.

In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule to inhibit, down-regulate, or reduce expression of a HCV gene, wherein the siNA molecule comprises one or more chemical modifications and each strand of the double-stranded siNA is independently about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 or more) nucleotides long. In one embodiment, the siNA molecule of the invention is a double stranded nucleic acid molecule comprising one or more chemical modifications, where each of the two fragments of the siNA molecule independently comprise about 15 to about 40 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 23, 33, 34, 35, 36, 37, 38, 39, or 40) nucleotides and where one of the strands comprises at least 15 nucleotides that are complementary to nucleotide sequence of HCV encoding RNA or a portion thereof. In a non-limiting example, each of the two fragments of the siNA molecule comprise about 21 nucleotides. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule comprising one or more chemical modifications, where each strand is about 21 nucleotide long and where about 19 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule, wherein at least two 3' terminal nucleotides of each fragment of the siNA molecule are not base-paired to the nucleotides of the other fragment of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule comprising one or more chemical modifications, where each strand is about 19 nucleotide long and where the nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule to form at least about 15 (e.g., 15, 16, 17, 18, or 19) base pairs, wherein one or both ends of the siNA molecule are blunt ends. In one embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule is a 2'-deoxy-pyrimidine nucleotide, such as a 2'-deoxy-thymidine. In another embodiment, all nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule of about 19

to about 25 base pairs having a sense region and an antisense region and comprising one or more chemical modifications, where about 19 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the HCV gene. In another embodiment, about 21 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the HCV gene. In any of the above embodiments, the 5'-end of the fragment comprising said antisense region can optionally include a phosphate group.

In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule that inhibits, down-regulates, or reduces expression of a HCV gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of HCV RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits, down-regulates, or reduces expression of a HCV gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of HCV RNA or a portion thereof, wherein the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits, down-regulates, or reduces expression of a HCV gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of HCV RNA that encodes a protein or portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar

modification. In one embodiment, each strand of the siNA molecule comprises about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides, wherein each strand comprises at least about 15 nucleotides that are complementary to the nucleotides of the other strand. In one embodiment, the siNA molecule is assembled from two oligonucleotide fragments, wherein one fragment comprises the nucleotide sequence of the antisense strand of the siNA molecule and a second fragment comprises nucleotide sequence of the sense region of the siNA molecule. In one embodiment, the sense strand is connected to the antisense strand via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker. In a further embodiment, the pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In another embodiment, the pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides. In still another embodiment, the pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and any purine nucleotides present in the antisense strand are 2'-deoxy purine nucleotides. In another embodiment, the antisense strand comprises one or more 2'-deoxy-2'-fluoro pyrimidine nucleotides and one or more 2'-O-methyl purine nucleotides. In another embodiment, the pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and any purine nucleotides present in the antisense strand are 2'-O-methyl purine nucleotides. In a further embodiment the sense strand comprises a 3'-end and a 5'-end, wherein a terminal cap moiety (e.g., an inverted deoxy abasic moiety or inverted deoxy nucleotide moiety such as inverted thymidine) is present at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the sense strand. In another embodiment, the antisense strand comprises a phosphorothioate internucleotide linkage at the 3' end of the antisense strand. In another embodiment, the antisense strand comprises a glyceryl modification at the 3' end. In another embodiment, the 5'-end of the antisense strand optionally includes a phosphate group.

In any of the above-described embodiments of a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a HCV gene, wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, each of the two strands of the siNA molecule can comprise about 15

to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides. In one embodiment, about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides of each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the siNA molecule. In another embodiment, about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides of each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the siNA molecule, wherein at least two 3' terminal nucleotides of each strand of the siNA molecule are not base-paired to the nucleotides of the other strand of the siNA molecule. In another embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule is a 2'-deoxy-pyrimidine, such as 2'-deoxy-thymidine. In one embodiment, each strand of the siNA molecule is base-paired to the complementary nucleotides of the other strand of the siNA molecule. In one embodiment, about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides of the antisense strand are base-paired to the nucleotide sequence of the HCV RNA or a portion thereof. In one embodiment, about 18 to about 25 (e.g., about 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides of the antisense strand are base-paired to the nucleotide sequence of the HCV RNA or a portion thereof.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a HCV gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of HCV RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the 5'-end of the antisense strand optionally includes a phosphate group.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a HCV gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of HCV RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a

majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence or a portion thereof of the antisense strand is complementary to a nucleotide sequence of the untranslated region or a portion thereof of the HCV RNA.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a HCV gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of HCV RNA or a portion thereof, wherein the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand, wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence of the antisense strand is complementary to a nucleotide sequence of the HCV RNA or a portion thereof that is present in the HCV RNA.

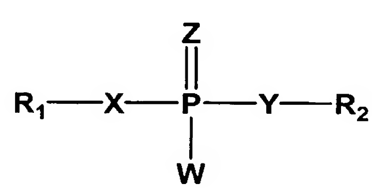
In one embodiment, the invention features a composition comprising a siNA molecule of the invention in a pharmaceutically acceptable carrier or diluent.

In a non-limiting example, the introduction of chemically-modified nucleotides into nucleic acid molecules provides a powerful tool in overcoming potential limitations of *in vivo* stability and bioavailability inherent to native RNA molecules that are delivered exogenously. For example, the use of chemically-modified nucleic acid molecules can enable a lower dose of a particular nucleic acid molecule for a given therapeutic effect since chemically-modified nucleic acid molecules tend to have a longer half-life in serum. Furthermore, certain chemical modifications can improve the bioavailability of nucleic acid molecules by targeting particular cells or tissues and/or improving cellular uptake of the nucleic acid molecule. Therefore, even if the activity of a chemically-modified nucleic acid molecule is reduced as compared to a native nucleic acid molecule, for example, when compared to an all-RNA nucleic acid molecule, the overall activity of the modified nucleic acid molecule can be greater than that of the native molecule due to improved stability and/or delivery of the molecule. Unlike native unmodified siNA, chemically-modified siNA can also minimize the possibility of activating interferon activity in humans.

In any of the embodiments of siNA molecules described herein, the antisense region of a siNA molecule of the invention can comprise a phosphorothioate internucleotide linkage at the 3'-end of said antisense region. In any of the embodiments of siNA molecules described herein, the antisense region can comprise about one to about five phosphorothioate internucleotide linkages at the 5'-end of said antisense region. In any of the embodiments of siNA molecules described herein, the 3'-terminal nucleotide overhangs of a siNA molecule of the invention can comprise ribonucleotides or deoxyribonucleotides that are chemically-modified at a nucleic acid sugar, base, or backbone. In any of the embodiments of siNA molecules described herein, the 3'-terminal nucleotide overhangs can comprise one or more universal base ribonucleotides. In any of the embodiments of siNA molecules described herein, the 3'-terminal nucleotide overhangs can comprise one or more acyclic nucleotides.

One embodiment of the invention provides an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the invention in a manner that allows expression of the nucleic acid molecule. Another embodiment of the invention provides a mammalian cell comprising such an expression vector. The mammalian cell can be a human cell. The siNA molecule of the expression vector can comprise a sense region and an antisense region. The antisense region can comprise sequence complementary to a RNA or DNA sequence encoding HCV and the sense region can comprise sequence complementary to the antisense region. The siNA molecule can comprise two distinct strands having complementary sense and antisense regions. The siNA molecule can comprise a single strand having complementary sense and antisense regions.

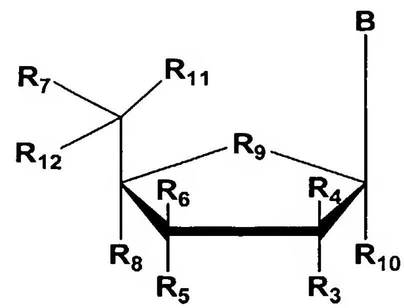
In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against HCV inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides comprising a backbone modified internucleotide linkage having Formula I:



wherein each R1 and R2 is independently any nucleotide, non-nucleotide, or polynucleotide which can be naturally-occurring or chemically-modified, each X and Y is independently O, S, N, alkyl, or substituted alkyl, each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, aralkyl, or acetyl and wherein W, X, Y, and Z are optionally not all O. In another embodiment, a backbone modification of the invention comprises a phosphonoacetate and/or thiophosphonoacetate internucleotide linkage (see for example Sheehan et al., 2003, Nucleic Acids Research, 31, 4109-4118).

The chemically-modified internucleotide linkages having Formula I, for example, wherein any Z, W, X, and/or Y independently comprises a sulphur atom, can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) chemically-modified internucleotide linkages having Formula I at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified internucleotide linkages having Formula I at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) purine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In another embodiment, a siNA molecule of the invention having internucleotide linkage(s) of Formula I also comprises a chemically-modified nucleotide or non-nucleotide having any of Formulae I-VII.

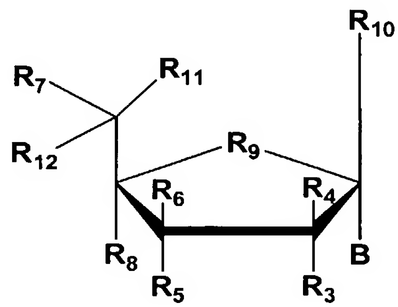
In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against HCV inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula II:



wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH2, S=O, CHF, or CF2, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA.

The chemically-modified nucleotide or non-nucleotide of Formula II can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotides or non-nucleotides of Formula II at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 5'-end of the sense strand, the antisense strand, or both strands. In anther non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 3'-end of the sense strand, the antisense strand, or both strands.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against HCV inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula III:



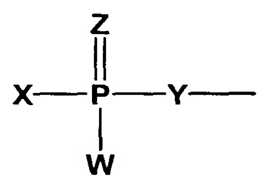
wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH2, S=O, CHF, or CF2, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be employed to be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA.

The chemically-modified nucleotide or non-nucleotide of Formula III can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotides or non-nucleotides of Formula III at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide(s) or non-nucleotide(s) of Formula III at the 5'-end of

the sense strand, the antisense strand, or both strands. In anther non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide or non-nucleotide of Formula III at the 3'-end of the sense strand, the antisense strand, or both strands.

In another embodiment, a siNA molecule of the invention comprises a nucleotide having Formula II or III, wherein the nucleotide having Formula II or III is in an inverted configuration. For example, the nucleotide having Formula II or III is connected to the siNA construct in a 3'-3', 3'-2', 2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against HCV inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises a 5'-terminal phosphate group having Formula IV:



wherein each X and Y is independently O, S, N, alkyl, substituted alkyl, or alkylhalo; wherein each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, aralkyl, alkylhalo, or acetyl; and wherein W, X, Y and Z are not all O.

In one embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complementary strand, for example, a strand complementary to a target RNA, wherein the siNA molecule comprises an all RNA siNA molecule. In another embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complementary strand wherein the siNA molecule also comprises about 1 to about 3 (*e.g.*, about 1, 2, or 3) nucleotide 3'-terminal nucleotide overhangs having about 1 to about 4 (*e.g.*, about 1, 2, 3, or 4) deoxyribonucleotides on the 3'-end of one or both strands. In another embodiment, a 5'-terminal phosphate group having Formula IV is present on the target-complementary strand of a siNA molecule of the invention, for example a siNA molecule having chemical modifications having any of Formulae I-VII.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against HCV inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more phosphorothioate internucleotide linkages. For example, in a non-limiting example, the invention features a chemically-modified short interfering nucleic acid (siNA) having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in one siNA strand. In yet another embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) individually having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in both siNA strands. The phosphorothioate internucleotide linkages can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more phosphorothioate internucleotide linkages at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) consecutive phosphorothioate internucleotide linkages at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine phosphorothioate internucleotide linkages in the sense strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) purine phosphorothioate internucleotide linkages in the sense strand, the antisense strand, or both strands.

In one embodiment, the invention features a siNA molecule, wherein the sense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or about one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3,

4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In another embodiment, the invention features a siNA molecule, wherein the sense strand comprises about 1 to about 5, specifically about 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5 or more, for example about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In one embodiment, the invention features a siNA molecule, wherein the antisense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or about one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or

more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3' and 5'-ends, being present in the same or different strand.

In another embodiment, the invention features a siNA molecule, wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5, for example about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule having about 1 to about 5 or more (specifically about 1, 2, 3, 4, 5 or more) phosphorothioate internucleotide linkages in each strand of the siNA molecule.

In another embodiment, the invention features a siNA molecule comprising 2'-5' internucleotide linkages. The 2'-5' internucleotide linkage(s) can be at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of one or both siNA sequence strands. In addition, the 2'-5' internucleotide linkage(s) can be present at various other positions within one or both siNA sequence strands, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a pyrimidine nucleotide in one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a purine nucleotide in one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage.

In another embodiment, a chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-modified, wherein each strand is independently about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides in length, wherein the duplex has about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) base pairs, and wherein the chemical modification comprises a structure having any of Formulae I-VII. For example, an exemplary chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein each strand consists of about 21 nucleotides, each having a 2-nucleotide 3'-terminal nucleotide overhang, and wherein the duplex has about 19 base pairs. In another embodiment, a siNA molecule of the invention comprises a single stranded hairpin structure, wherein the siNA is about 36 to about 70 (*e.g.*, about 36, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) base pairs, and wherein the siNA can include a chemical modification comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 42 to about 50 (*e.g.*, about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms a hairpin structure having about 19 to about 21 (e.g., 19, 20, or 21) base pairs and a 2-nucleotide 3'-terminal nucleotide overhang. In another embodiment, a linear hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of

the siNA molecule is biodegradable. For example, a linear hairpin siNA molecule of the invention is designed such that degradation of the loop portion of the siNA molecule *in vivo* can generate a double-stranded siNA molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

In another embodiment, a siNA molecule of the invention comprises a hairpin structure, wherein the siNA is about 25 to about 50 (*e.g.*, about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides in length having about 3 to about 25 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 25 to about 35 (*e.g.*, about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35) nucleotides that is chemically-modified with one or more chemical modifications having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms a hairpin structure having about 3 to about 25 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs and a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV). In another embodiment, a linear hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. In one embodiment, a linear hairpin siNA molecule of the invention comprises a loop portion comprising a non-nucleotide linker.

In another embodiment, a siNA molecule of the invention comprises an asymmetric hairpin structure, wherein the siNA is about 25 to about 50 (*e.g.*, about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides in length having about 3 to about 25 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 25 to about 35 (*e.g.*, about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35) nucleotides that is chemically-modified with one or more chemical modifications having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms an

asymmetric hairpin structure having about 3 to about 25 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs and a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV). In one embodiment, an asymmetric hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. In another embodiment, an asymmetric hairpin siNA molecule of the invention comprises a loop portion comprising a non-nucleotide linker.

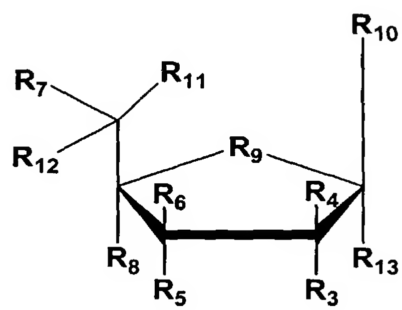
In another embodiment, a siNA molecule of the invention comprises an asymmetric double stranded structure having separate polynucleotide strands comprising sense and antisense regions, wherein the antisense region is about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides in length, wherein the sense region is about 3 to about 25 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides in length, wherein the sense region and the antisense region have at least 3 complementary nucleotides, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises an asymmetric double stranded structure having separate polynucleotide strands comprising sense and antisense regions, wherein the antisense region is about 18 to about 23 (*e.g.*, about 18, 19, 20, 21, 22, or 23) nucleotides in length and wherein the sense region is about 3 to about 15 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) nucleotides in length, wherein the sense region the antisense region have at least 3 complementary nucleotides, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. In another embodiment, the asymmetric double stranded siNA molecule can also have a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV).

In another embodiment, a siNA molecule of the invention comprises a circular nucleic acid molecule, wherein the siNA is about 38 to about 70 (*e.g.*, about 38, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) base pairs, and wherein the siNA can include a chemical modification, which comprises a structure having any of

Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a circular oligonucleotide having about 42 to about 50 (*e.g.*, about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the circular oligonucleotide forms a dumbbell shaped structure having about 19 base pairs and 2 loops.

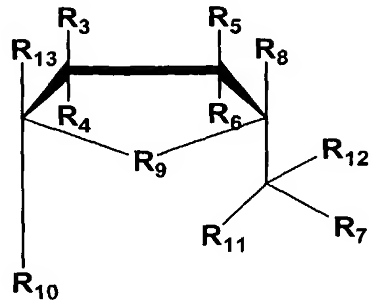
In another embodiment, a circular siNA molecule of the invention contains two loop motifs, wherein one or both loop portions of the siNA molecule is biodegradable. For example, a circular siNA molecule of the invention is designed such that degradation of the loop portions of the siNA molecule *in vivo* can generate a double-stranded siNA molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

In one embodiment, a siNA molecule of the invention comprises at least one (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) abasic moiety, for example a compound having Formula V:



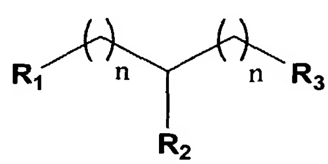
wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH2, S=O, CHF, or CF2.

In one embodiment, a siNA molecule of the invention comprises at least one (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) inverted abasic moiety, for example a compound having Formula VI:



wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH2, S=O, CHF, or CF2, and either R2, R3, R8 or R13 serve as points of attachment to the siNA molecule of the invention.

In another embodiment, a siNA molecule of the invention comprises at least one (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) substituted polyalkyl moieties, for example a compound having Formula VII:



wherein each n is independently an integer from 1 to 12, each R1, R2 and R3 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or a group having Formula I, and R1, R2 or R3 serves as points of attachment to the siNA molecule of the invention.

In another embodiment, the invention features a compound having Formula VII, wherein R1 and R2 are hydroxyl (OH) groups, n = 1, and R3 comprises O and is the

point of attachment to the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both strands of a double-stranded siNA molecule of the invention or to a single-stranded siNA molecule of the invention. This modification is referred to herein as "glyceryl" (for example modification 6 in **Figure 10**).

In another embodiment, a chemically modified nucleoside or non-nucleoside (e.g. a moiety having any of Formula V, VI or VII) of the invention is at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of a siNA molecule of the invention. For example, chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) can be present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense strand, the sense strand, or both antisense and sense strands of the siNA molecule. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the terminal position of the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the two terminal positions of the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the penultimate position of the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In addition, a moiety having Formula VII can be present at the 3'-end or the 5'-end of a hairpin siNA molecule as described herein.

In another embodiment, a siNA molecule of the invention comprises an abasic residue having Formula V or VI, wherein the abasic residue having Formula VI or VI is connected to the siNA construct in a 3'-3', 3'-2', 2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

In one embodiment, a siNA molecule of the invention comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) locked nucleic acid (LNA) nucleotides, for

example, at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

In another embodiment, a siNA molecule of the invention comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) acyclic nucleotides, for example, at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said sense region are 2'-deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all)

purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), wherein any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said sense region are 2'-deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and

wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said antisense region are 2'-deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention capable of mediating RNA interference (RNAi) against HCV inside a cell or reconstituted *in vitro* system comprising a sense region, wherein one or more pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and one or more purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), and an antisense region, wherein one or more pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro

pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and one or more purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides). The sense region and/or the antisense region can have a terminal cap modification, such as any modification described herein or shown in **Figure 10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense and/or antisense sequence. The sense and/or antisense region can optionally further comprise a 3'-terminal nucleotide overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxynucleotides. The overhang nucleotides can further comprise one or more (e.g., about 1, 2, 3, 4 or more) phosphorothioate, phosphonoacetate, and/or thiophosphonoacetate internucleotide linkages. Non-limiting examples of these chemically-modified siNAs are shown in **Figures 4 and 5** and **Tables III and IV** herein. In any of these described embodiments, the purine nucleotides present in the sense region are alternatively 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides) and one or more purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides). Also, in any of these embodiments, one or more purine nucleotides present in the sense region are alternatively purine ribonucleotides (e.g., wherein all purine nucleotides are purine ribonucleotides or alternately a plurality of purine nucleotides are purine ribonucleotides) and any purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides). Additionally, in any of these embodiments, one or more purine nucleotides present in the sense region and/or present in the antisense region are alternatively selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides (e.g., wherein all purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides or alternately a plurality of purine nucleotides are selected from the group

consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides).

In another embodiment, any modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for example Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984). As such, chemically modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, are resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi. Non-limiting examples of nucleotides having a northern configuration include locked nucleic acid (LNA) nucleotides (e.g., 2′-O, 4′-C-methylene-(D-ribofuranosyl) nucleotides); 2'-methoxyethoxy (MOE) nucleotides; 2'-methyl-thio-ethyl, 2'-deoxy-2'-fluoro nucleotides, 2'-deoxy-2'-chloro nucleotides, 2'-azido nucleotides, and 2'-O-methyl nucleotides.

In one embodiment, the sense strand of a double stranded siNA molecule of the invention comprises a terminal cap moiety, (see for example **Figure 10**) such as an inverted deoxyabaisc moiety, at the 3'-end, 5'-end, or both 3' and 5'-ends of the sense strand.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid molecule (siNA) capable of mediating RNA interference (RNAi) against HCV inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises a conjugate covalently attached to the chemically-modified siNA molecule. Non-limiting examples of conjugates contemplated by the invention include conjugates and ligands described in Vargeese *et al.*, USSN 10/427,160, filed April 30, 2003, incorporated by reference herein in its entirety, including the drawings. In another embodiment, the conjugate is covalently attached to the chemically-modified siNA molecule via a biodegradable linker. In one embodiment, the conjugate molecule is attached at the 3'-end of either the sense strand, the antisense strand, or both strands of

the chemically-modified siNA molecule. In another embodiment, the conjugate molecule is attached at the 5'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule. In yet another embodiment, the conjugate molecule is attached both the 3'-end and 5'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule, or any combination thereof. In one embodiment, a conjugate molecule of the invention comprises a molecule that facilitates delivery of a chemically-modified siNA molecule into a biological system, such as a cell. In another embodiment, the conjugate molecule attached to the chemically-modified siNA molecule is a polyethylene glycol, human serum albumin, or a ligand for a cellular receptor that can mediate cellular uptake. Examples of specific conjugate molecules contemplated by the instant invention that can be attached to chemically-modified siNA molecules are described in Vargeese *et al.*, U.S. Serial No. 10/201,394, filed July 22, 2002 incorporated by reference herein. The type of conjugates used and the extent of conjugation of siNA molecules of the invention can be evaluated for improved pharmacokinetic profiles, bioavailability, and/or stability of siNA constructs while at the same time maintaining the ability of the siNA to mediate RNAi activity. As such, one skilled in the art can screen siNA constructs that are modified with various conjugates to determine whether the siNA conjugate complex possesses improved properties while maintaining the ability to mediate RNAi, for example in animal models as are generally known in the art.

In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule of the invention, wherein the siNA further comprises a nucleotide, non-nucleotide, or mixed nucleotide/non-nucleotide linker that joins the sense region of the siNA to the antisense region of the siNA. In one embodiment, a nucleotide linker of the invention can be a linker of ≥ 2 nucleotides in length, for example about 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length. In another embodiment, the nucleotide linker can be a nucleic acid aptamer. By "aptamer" or "nucleic acid aptamer" as used herein is meant a nucleic acid molecule that binds specifically to a target molecule wherein the nucleic acid molecule has sequence that comprises a sequence recognized by the target molecule in its natural setting. Alternately, an aptamer can be a nucleic acid molecule that binds to a target molecule where the target molecule does not naturally bind to a nucleic acid. The target molecule can be any molecule of interest. For example, the aptamer can be used to bind to a ligand-binding domain of a protein, thereby preventing interaction of

the naturally occurring ligand with the protein. This is a non-limiting example and those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art. (See, for example, Gold *et al.,* 1995, *Annu. Rev. Biochem.,* 64, 763; Brody and Gold, 2000, *J. Biotechnol.,* 74, 5; Sun, 2000, *Curr. Opin. Mol. Ther.,* 2, 100; Kusser, 2000, *J. Biotechnol.,* 74, 27; Hermann and Patel, 2000, *Science,* 287, 820; and Jayasena, 1999, *Clinical Chemistry,* 45, 1628.)

In yet another embodiment, a non-nucleotide linker of the invention comprises abasic nucleotide, polyether, polyamine, polyamide, peptide, carbohydrate, lipid, polyhydrocarbon, or other polymeric compounds (e.g. polyethylene glycols such as those having between 2 and 100 ethylene glycol units). Specific examples include those described by Seela and Kaiser, *Nucleic Acids Res.* 1990, *18*:6353 and *Nucleic Acids Res.* 1987, *15*:3113; Cload and Schepartz, *J. Am. Chem. Soc.* 1991, *113*:6324; Richardson and Schepartz, *J. Am. Chem. Soc.* 1991, *113*:5109; Ma *et al., Nucleic Acids Res.* 1993, *21*:2585 and *Biochemistry* 1993, *32*:1751; Durand *et al., Nucleic Acids Res.* 1990, *18*:6353; McCurdy *et al., Nucleosides & Nucleotides* 1991, *10*:287; Jschke *et al., Tetrahedron Lett.* 1993, *34*:301; Ono *et al., Biochemistry* 1991, *30*:9914; Arnold *et al.,* International Publication No. WO 89/02439; Usman *et al.,* International Publication No. WO 95/06731; Dudycz *et al.,* International Publication No. WO 95/11910 and Ferentz and Verdine, *J. Am. Chem. Soc.* 1991, *113*:4000, all hereby incorporated by reference herein. A "non-nucleotide" further means any group or compound that can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound can be abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine, for example at the C1 position of the sugar.

In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) inside a cell or reconstituted *in vitro* system, wherein one or both strands of the siNA molecule that are assembled from two separate oligonucleotides do not comprise any ribonucleotides. For example, a siNA molecule can be assembled from a single oligonculeotide where the sense and antisense regions of the siNA comprise separate oligonucleotides that do not have any ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotides. In another example, a siNA molecule can be assembled from a single oligonculeotide

where the sense and antisense regions of the siNA are linked or circularized by a nucleotide or non-nucleotide linker as described herein, wherein the oligonucleotide does not have any ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotide. Applicant has surprisingly found that the presense of ribonucleotides (e.g., nucleotides having a 2'-hydroxyl group) within the siNA molecule is not required or essential to support RNAi activity. As such, in one embodiment, all positions within the siNA can include chemically modified nucleotides and/or non-nucleotides such as nucleotides and or non-nucleotides having Formula I, II, III, IV, V, VI, or VII or any combination thereof to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted *in vitro* system comprising a single stranded polynucleotide having complementarity to a target nucleic acid sequence. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group and a 3'-terminal phosphate group (e.g., a 2',3'-cyclic phosphate). In another embodiment, the single stranded siNA molecule of the invention comprises about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides. In yet another embodiment, the single stranded siNA molecule of the invention comprises one or more chemically modified nucleotides or non-nucleotides described herein. For example, all the positions within the siNA molecule can include chemically-modified nucleotides such as nucleotides having any of Formulae I-VII, or any combination thereof to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted *in vitro* system comprising a single stranded polynucleotide having complementarity to a target nucleic acid sequence, wherein one or more pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g.,

wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in **Figure 10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence. The siNA optionally further comprises about 1 to about 4 or more (e.g., about 1, 2, 3, 4 or more) terminal 2'-deoxynucleotides at the 3'-end of the siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, 4 or more) phosphorothioate, phosphonoacetate, and/or thiophosphonoacetate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group. In any of these embodiments, any purine nucleotides present in the antisense region are alternatively 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides). Also, in any of these embodiments, any purine nucleotides present in the siNA (i.e., purine nucleotides present in the sense and/or antisense region) can alternatively be locked nucleic acid (LNA) nucleotides (e.g., wherein all purine nucleotides are LNA nucleotides or alternately a plurality of purine nucleotides are LNA nucleotides). Also, in any of these embodiments, any purine nucleotides present in the siNA are alternatively 2'-methoxyethyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-methoxyethyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-methoxyethyl purine nucleotides). In another embodiment, any modified nucleotides present in the single stranded siNA molecules of the invention comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for example Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984). As such, chemically modified nucleotides present in the single stranded siNA molecules of the invention are preferably resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi.

In one embodiment, a siNA molecule of the invention comprises chemically modified nucleotides or non-nucleotides (e.g., having any of Formulae I-VII, such as 2'-deoxy, 2'-deoxy-2'-fluoro, or 2'-O-methyl nucleotides) at alternating positions within one or more strands or regions of the siNA molecule. For example, such chemical

modifications can be introduced at every other position of a RNA based siNA molecule, starting at either the first or second nucleotide from the 3'-end or 5'-end of the siNA. In a non-limiting example, a double stranded siNA molecule of the invention in which each strand of the siNA is 21 nucleotides in length is featured wherein positions 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21 of each strand are chemically modified (e.g., with compounds having any of Formulae 1-VII, such as such as 2'-deoxy, 2'-deoxy-2'-fluoro, or 2'-O-methyl nucleotides). In another non-limiting example, a double stranded siNA molecule of the invention in which each strand of the siNA is 21 nucleotides in length is featured wherein positions 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 of each strand are chemically modified (e.g., with compounds having any of Formulae 1-VII, such as such as 2'-deoxy, 2'-deoxy-2'-fluoro, or 2'-O-methyl nucleotides). Such siNA molecules can further comprise terminal cap moieties and/or backbone modifications as described herein.

In one embodiment, the invention features a method for modulating the expression of a HCV gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the HCV gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate (e.g., inhibit) the expression of the HCV gene in the cell.

In one embodiment, the invention features a method for modulating the expression of a HCV gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the HCV gene and wherein the sense strand sequence of the siNA comprises a sequence identical or substantially similar to the sequence of the target RNA; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate (e.g., inhibit) the expression of the HCV gene in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one HCV gene within a cell comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the HCV genes; and (b) introducing the siNA molecules into a cell under conditions suitable to modulate (e.g., inhibit) the expression of the HCV genes in the cell.

In another embodiment, the invention features a method for modulating the expression of two or more HCV genes within a cell comprising: (a) synthesizing one or more siNA molecules of the invention, which can be chemically-modified, wherein the siNA strands comprise sequences complementary to RNA of the HCV genes and wherein the sense strand sequences of the siNAs comprise sequences identical or substantially similar to the sequences of the target RNAs; and (b) introducing the siNA molecules into a cell under conditions suitable to modulate (e.g., inhibit) the expression of the HCV genes in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one HCV gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the HCV gene and wherein the sense strand sequence of the siNA comprises a sequence identical or substantially similar to the sequences of the target RNAs; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate (e.g., inhibit) the expression of the HCV genes in the cell.

In one embodiment, siNA molecules of the invention are used as reagents in *ex vivo* applications. For example, siNA reagents are introduced into tissue or cells that are transplanted into a subject for therapeutic effect. The cells and/or tissue can be derived from an organism or subject that later receives the explant, or can be derived from another organism or subject prior to transplantation. The siNA molecules can be used to modulate the expression of one or more genes in the cells or tissue, such that the cells or tissue obtain a desired phenotype or are able to perform a function when transplanted in vivo. In one embodiment, certain target cells from a patient are extracted. These extracted cells are contacted with siNAs targeting a specific nucleotide sequence within the cells under conditions suitable for uptake of the siNAs by these cells (e.g. using delivery reagents such as cationic lipids, liposomes and the like or using techniques such as electroporation to facilitate the delivery of siNAs into cells). The cells are then reintroduced back into the same patient or other patients. In one embodiment, the invention features a method of modulating the expression of a HCV gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the HCV gene; and (b) introducing the siNA molecule into a

cell of the tissue explant derived from a particular organism under conditions suitable to modulate (e.g., inhibit) the expression of the HCV gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate (e.g., inhibit) the expression of the HCV gene in that organism.

In one embodiment, the invention features a method of modulating the expression of a HCV gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the HCV gene and wherein the sense strand sequence of the siNA comprises a sequence identical or substantially similar to the sequence of the target RNA; and (b) introducing the siNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate (e.g., inhibit) the expression of the HCV gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate (e.g., inhibit) the expression of the HCV gene in that organism.

In another embodiment, the invention features a method of modulating the expression of more than one HCV gene in a tissue explant comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the HCV genes; and (b) introducing the siNA molecules into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate (e.g., inhibit) the expression of the HCV genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate (e.g., inhibit) the expression of the HCV genes in that organism.

In one embodiment, the invention features a method of modulating the expression of a HCV gene in a subject or organism comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the HCV gene; and (b) introducing the siNA molecule into the subject or organism under conditions suitable to modulate (e.g.,

inhibit) the expression of the HCV gene in the subject or organism. The level of HCV protein or RNA can be determined using various methods well-known in the art.

In another embodiment, the invention features a method of modulating the expression of more than one HCV gene in a subject or organism comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the HCV genes; and (b) introducing the siNA molecules into the subject or organism under conditions suitable to modulate (e.g., inhibit) the expression of the HCV genes in the subject or organism. The level of HCV protein or RNA can be determined as is known in the art.

In one embodiment, the invention features a method for modulating the expression of a HCV gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the HCV gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate (e.g., inhibit) the expression of the HCV gene in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one HCV gene within a cell comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the HCV gene; and (b) contacting the cell *in vitro* or *in vivo* with the siNA molecule under conditions suitable to modulate (e.g., inhibit) the expression of the HCV genes in the cell.

In one embodiment, the invention features a method of modulating the expression of a HCV gene in a tissue explant (e.g., a liver transplant) comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the HCV gene; and (b) contacting a cell of the tissue explant derived from a particular subject or organism with the siNA molecule under conditions suitable to modulate (e.g., inhibit) the expression of the HCV gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the subject or organism the tissue was derived from or into another subject or organism under conditions suitable to modulate (e.g., inhibit) the expression of the HCV gene in that subject or organism.

In another embodiment, the invention features a method of modulating the expression of more than one HCV gene in a tissue explant (e.g., a liver transplant) comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the HCV gene; and (b) introducing the siNA molecules into a cell of the tissue explant derived from a particular subject or organism under conditions suitable to modulate (e.g., inhibit) the expression of the HCV genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the subject or organism the tissue was derived from or into another subject or organism under conditions suitable to modulate (e.g., inhibit) the expression of the HCV genes in that subject or organism.

In one embodiment, the invention features a method of modulating the expression of a HCV gene in a subject or organism comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the HCV gene; and (b) introducing the siNA molecule into the subject or organism under conditions suitable to modulate (e.g., inhibit) the expression of the HCV gene in the subject or organism.

In another embodiment, the invention features a method of modulating the expression of more than one HCV gene in a subject or organism comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the HCV gene; and (b) introducing the siNA molecules into the subject or organism under conditions suitable to modulate (e.g., inhibit) the expression of the HCV genes in the subject or organism.

In one embodiment, the invention features a method of modulating the expression of a HCV gene in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate (e.g., inhibit) the expression of the HCV gene in the subject or organism.

In one embodiment, the invention features a method for treating or preventing HCV infection in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate (e.g.,

inhibit) the expression of an inhibitor of HCV gene expression in the subject or organism.

In one embodiment, the invention features a method for treating or preventing liver failure in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate (e.g., inhibit) the expression of an inhibitor of HCV gene expression in the subject or organism.

In one embodiment, the invention features a method for treating or preventing hepatocellular carcinoma in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate (e.g., inhibit) the expression of an inhibitor of HCV gene expression in the subject or organism.

In one embodiment, the invention features a method for treating or preventing cirrhosis in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate (e.g., inhibit) the expression of an inhibitor of HCV gene expression in the subject or organism.

In another embodiment, the invention features a method of modulating the expression of more than one HCV gene in a subject or organism comprising contacting the subject or organism with one or more siNA molecules of the invention under conditions suitable to modulate (e.g., inhibit) the expression of the HCV genes in the subject or organism.

The siNA molecules of the invention can be designed to down regulate or inhibit target (e.g., HCV) gene expression through RNAi targeting of a variety of RNA molecules. In one embodiment, the siNA molecules of the invention are used to target various RNAs corresponding to a target gene. Non-limiting examples of such RNAs include messenger RNA (mRNA), alternate RNA splice variants of target gene(s), post-transcriptionally modified RNA of target gene(s), pre-mRNA of target gene(s), and/or RNA templates. If alternate splicing produces a family of transcripts that are distinguished by usage of appropriate exons, the instant invention can be used to inhibit gene expression through the appropriate exons to specifically inhibit or to distinguish among the functions of gene family members. For example, a protein that contains an alternatively spliced transmembrane domain can be expressed in both membrane bound

and secreted forms. Use of the invention to target the exon containing the transmembrane domain can be used to determine the functional consequences of pharmaceutical targeting of membrane bound as opposed to the secreted form of the protein. Non-limiting examples of applications of the invention relating to targeting these RNA molecules include therapeutic pharmaceutical applications, pharmaceutical discovery applications, molecular diagnostic and gene function applications, and gene mapping, for example using single nucleotide polymorphism mapping with siNA molecules of the invention. Such applications can be implemented using known gene sequences or from partial sequences available from an expressed sequence tag (EST).

In another embodiment, the siNA molecules of the invention are used to target conserved sequences corresponding to a gene family or gene families such as HCV family genes (e.g., all known HCV strains, groups of related HCV strains, or groups of divergent HCV strains). As such, siNA molecules targeting multiple HCV targets can provide increased therapeutic effect. In addition, siNA can be used to characterize pathways of gene function in a variety of applications. For example, the present invention can be used to inhibit the activity of target gene(s) in a pathway to determine the function of uncharacterized gene(s) in gene function analysis, mRNA function analysis, or translational analysis. The invention can be used to determine potential target gene pathways involved in various diseases and conditions toward pharmaceutical development. The invention can be used to understand pathways of gene expression involved in, for example proliferative diseases, disorders and conditions.

In one embodiment, siNA molecule(s) and/or methods of the invention are used to down regulate the expression of gene(s) that encode RNA referred to by Genbank Accession, for example, HCV genes encoding RNA sequence(s) referred to herein by Genbank Accession number, for example, Genbank Accession Nos. shown in **Table I**.

In one embodiment, the invention features a method comprising: (a) generating a library of siNA constructs having a predetermined complexity; and (b) assaying the siNA constructs of (a) above, under conditions suitable to determine RNAi target sites within the target RNA sequence. In one embodiment, the siNA molecules of (a) have strands of a fixed length, for example, about 23 nucleotides in length. In another embodiment, the siNA molecules of (a) are of differing length, for example having strands of about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30)

nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. In another embodiment, fragments of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNAse protection assays, to determine the most suitable target site(s) within the target RNA sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by cellular expression in *in vivo* systems.

In one embodiment, the invention features a method comprising: (a) generating a randomized library of siNA constructs having a predetermined complexity, such as of 4^N, where N represents the number of base paired nucleotides in each of the siNA construct strands (*eg.* for a siNA construct having 21 nucleotide sense and antisense strands with 19 base pairs, the complexity would be 4^{19}); and (b) assaying the siNA constructs of (a) above, under conditions suitable to determine RNAi target sites within the target HCV RNA sequence. In another embodiment, the siNA molecules of (a) have strands of a fixed length, for example about 23 nucleotides in length. In yet another embodiment, the siNA molecules of (a) are of differing length, for example having strands of about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described in Example 6 herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. In another embodiment, fragments of HCV RNA are analyzed for detectable levels of cleavage, for example, by gel electrophoresis, northern blot analysis, or RNAse protection assays, to determine the most suitable target site(s) within the target HCV RNA sequence. The target HCV RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by cellular expression in *in vivo* systems.

In another embodiment, the invention features a method comprising: (a) analyzing the sequence of a RNA target encoded by a target gene; (b) synthesizing one or more sets of siNA molecules having sequence complementary to one or more regions of the RNA of (a); and (c) assaying the siNA molecules of (b) under conditions suitable to determine RNAi targets within the target RNA sequence. In one embodiment, the siNA molecules of (b) have strands of a fixed length, for example about 23 nucleotides in length. In

another embodiment, the siNA molecules of (b) are of differing length, for example having strands of about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. Fragments of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNAse protection assays, to determine the most suitable target site(s) within the target RNA sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by expression in *in vivo* systems.

By "target site" is meant a sequence within a target RNA that is "targeted" for cleavage mediated by a siNA construct which contains sequences within its antisense region that are complementary to the target sequence.

By "detectable level of cleavage" is meant cleavage of target RNA (and formation of cleaved product RNAs) to an extent sufficient to discern cleavage products above the background of RNAs produced by random degradation of the target RNA. Production of cleavage products from 1-5% of the target RNA is sufficient to detect above the background for most methods of detection.

In one embodiment, the invention features a composition comprising a siNA molecule of the invention, which can be chemically-modified, in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a pharmaceutical composition comprising siNA molecules of the invention, which can be chemically-modified, targeting one or more genes in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a method for diagnosing a disease or condition in a subject comprising administering to the subject a composition of the invention under conditions suitable for the diagnosis of the disease or condition in the subject. In another embodiment, the invention features a method for treating or preventing a disease or condition in a subject, comprising administering to the subject a composition of the invention under conditions suitable for the treatment or prevention of the disease or condition in the subject, alone or in conjunction with one or more other therapeutic compounds. In yet another embodiment, the invention features a method for inhibiting, reducing or preventing HCV infection, liver failure, hepatocellular

carcinoma, cirrhosis in a subject or organism comprising administering to the subject a composition of the invention under conditions suitable for inhibiting, reducing or preventing HCV infection, liver failure, hepatocellular carcinoma, cirrhosis in the subject or organism.

In another embodiment, the invention features a method for validating a HCV gene target, comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands includes a sequence complementary to RNA of a HCV target gene; (b) introducing the siNA molecule into a cell, tissue, subject, or organism under conditions suitable for modulating expression of the HCV target gene in the cell, tissue, subject, or organism; and (c) determining the function of the gene by assaying for any phenotypic change in the cell, tissue, subject, or organism.

In another embodiment, the invention features a method for validating a HCV target comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands includes a sequence complementary to RNA of a HCV target gene; (b) introducing the siNA molecule into a biological system under conditions suitable for modulating expression of the HCV target gene in the biological system; and (c) determining the function of the gene by assaying for any phenotypic change in the biological system.

By “biological system” is meant, material, in a purified or unpurified form, from biological sources, including but not limited to human or animal, wherein the system comprises the components required for RNAi activity. The term “biological system” includes, for example, a cell, tissue, subject, or organism, or extract thereof. The term biological system also includes reconstituted RNAi systems that can be used in an *in vitro* setting.

By "phenotypic change" is meant any detectable change to a cell that occurs in response to contact or treatment with a nucleic acid molecule of the invention (e.g., siNA). Such detectable changes include, but are not limited to, changes in shape, size, proliferation, motility, protein expression or RNA expression or other physical or chemical changes as can be assayed by methods known in the art. The detectable change can also include expression of reporter genes/molecules such as Green Florescent Protein

(GFP) or various tags that are used to identify an expressed protein or any other cellular component that can be assayed.

In one embodiment, the invention features a kit containing a siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of a HCV target gene in a biological system, including, for example, in a cell, tissue, subject, or organism. In another embodiment, the invention features a kit containing more than one siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of more than one HCV target gene in a biological system, including, for example, in a cell, tissue, subject, or organism.

In one embodiment, the invention features a cell containing one or more siNA molecules of the invention, which can be chemically-modified. In another embodiment, the cell containing a siNA molecule of the invention is a mammalian cell. In yet another embodiment, the cell containing a siNA molecule of the invention is a human cell.

In one embodiment, the synthesis of a siNA molecule of the invention, which can be chemically-modified, comprises: (a) synthesis of two complementary strands of the siNA molecule; (b) annealing the two complementary strands together under conditions suitable to obtain a double-stranded siNA molecule. In another embodiment, synthesis of the two complementary strands of the siNA molecule is by solid phase oligonucleotide synthesis. In yet another embodiment, synthesis of the two complementary strands of the siNA molecule is by solid phase tandem oligonucleotide synthesis.

In one embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing a first oligonucleotide sequence strand of the siNA molecule, wherein the first oligonucleotide sequence strand comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of the second oligonucleotide sequence strand of the siNA; (b) synthesizing the second oligonucleotide sequence strand of siNA on the scaffold of the first oligonucleotide sequence strand, wherein the second oligonucleotide sequence strand further comprises a chemical moiety than can be used to purify the siNA duplex; (c) cleaving the linker molecule of (a) under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex; and (d) purifying the siNA duplex utilizing the chemical moiety of the second oligonucleotide sequence strand. In one embodiment, cleavage of the linker molecule in (c) above takes place during deprotection of the oligonucleotide, for

example, under hydrolysis conditions using an alkylamine base such as methylamine. In one embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand can comprise similar reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place concomitantly. In another embodiment, the chemical moiety of (b) that can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group, which can be employed in a trityl-on synthesis strategy as described herein. In yet another embodiment, the chemical moiety, such as a dimethoxytrityl group, is removed during purification, for example, using acidic conditions.

In a further embodiment, the method for siNA synthesis is a solution phase synthesis or hybrid phase synthesis wherein both strands of the siNA duplex are synthesized in tandem using a cleavable linker attached to the first sequence which acts a scaffold for synthesis of the second sequence. Cleavage of the linker under conditions suitable for hybridization of the separate siNA sequence strands results in formation of the double-stranded siNA molecule.

In another embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing one oligonucleotide sequence strand of the siNA molecule, wherein the sequence comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of another oligonucleotide sequence; (b) synthesizing a second oligonucleotide sequence having complementarity to the first sequence strand on the scaffold of (a), wherein the second sequence comprises the other strand of the double-stranded siNA molecule and wherein the second sequence further comprises a chemical moiety than can be used to isolate the attached oligonucleotide sequence; (c) purifying the product of (b) utilizing the chemical moiety of the second oligonucleotide sequence strand under conditions suitable for isolating the full-length sequence comprising both siNA oligonucleotide strands connected by the cleavable linker and under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex. In one embodiment, cleavage of the linker molecule in (c) above takes place during deprotection of the oligonucleotide, for example, under

hydrolysis conditions. In another embodiment, cleavage of the linker molecule in (c) above takes place after deprotection of the oligonucleotide. In another embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand can comprise similar reactivity or differing reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place either concomitantly or sequentially. In one embodiment, the chemical moiety of (b) that can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group.

In another embodiment, the invention features a method for making a double-stranded siNA molecule in a single synthetic process comprising: (a) synthesizing an oligonucleotide having a first and a second sequence, wherein the first sequence is complementary to the second sequence, and the first oligonucleotide sequence is linked to the second sequence via a cleavable linker, and wherein a terminal 5'-protecting group, for example, a 5'-O-dimethoxytrityl group (5'-O-DMT) remains on the oligonucleotide having the second sequence; (b) deprotecting the oligonucleotide whereby the deprotection results in the cleavage of the linker joining the two oligonucleotide sequences; and (c) purifying the product of (b) under conditions suitable for isolating the double-stranded siNA molecule, for example using a trityl-on synthesis strategy as described herein.

In another embodiment, the method of synthesis of siNA molecules of the invention comprises the teachings of Scaringe *et al.*, US Patent Nos. 5,889,136; 6,008,400; and 6,111,086, incorporated by reference herein in their entirety.

In one embodiment, the invention features siNA constructs that mediate RNAi against HCV, wherein the siNA construct comprises one or more chemical modifications, for example, one or more chemical modifications having any of Formulae I-VII or any combination thereof that increases the nuclease resistance of the siNA construct.

In another embodiment, the invention features a method for generating siNA molecules with increased nuclease resistance comprising (a) introducing nucleotides

having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased nuclease resistance.

In another embodiment, the invention features a method for generating siNA molecules with improved toxicologic profiles (e.g., have attenuated or no immunstimulatory properties) comprising (a) introducing nucleotides having any of Formula I-VII (e.g., siNA motifs referred to in **Table IV**) or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved toxicologic profiles.

In another embodiment, the invention features a method for generating siNA molecules that do not stimulate an interferon response (e.g., no interferon response or attenuated interferon response) in a cell, subject, or organism, comprising (a) introducing nucleotides having any of Formula I-VII (e.g., siNA motifs referred to in **Table IV**) or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules that do not stimulate an interferon response.

By "improved toxicologic profile", is meant that the chemically modified siNA construct exhibits decreased toxicity in a cell, subject, or organism compared to an unmodified siNA or siNA molecule having fewer modifications or modifications that are less effective in imparting improved toxicology. In a non-limiting example, siNA molecules with improved toxicologic profiles are associated with a decreased or attenuated immunostimulatory response in a cell, subject, or organism compared to an unmodified siNA or siNA molecule having fewer modifications or modifications that are less effective in imparting improved toxicology. In one embodiment, a siNA molecule with an improved toxicological profile comprises no ribonucleotides. In one embodiment, a siNA molecule with an improved toxicological profile comprises less than 5 ribonucleotides (e.g., 1, 2, 3, or 4 ribonucleotides). In one embodiment, a siNA molecule with an improved toxicological profile comprises Stab 7, Stab 8, Stab 11, Stab 12, Stab 13, Stab 16, Stab 17, Stab 18, Stab 19, Stab 20, Stab 23, Stab 24, Stab 25, Stab 26, Stab 27, Stab 28, Stab 29, Stab 30, Stab 31, Stab 32 or any combination thereof (see **Table IV**). In one embodiment, the level of immunostimulatory response associated with a given siNA molecule can be measured as is known in the art, for example by

nucleotides having any of Formula I-VII (e.g., siNA motifs referred to in **Table IV**) or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules that do not stimulate an interferon response.

By "improved toxicologic profile", is meant that the chemically modified siNA construct exhibits decreased toxicity in a cell, subject, or organism compared to an unmodified siNA or siNA molecule having fewer modifications or modifications that are less effective in imparting improved toxicology. In a non-limiting example, siNA molecules with improved toxicologic profiles are associated with a decreased or attenuated immunostimulatory response in a cell, subject, or organism compared to an unmodified siNA or siNA molecule having fewer modifications or modifications that are less effective in imparting improved toxicology. In one embodiment, a siNA molecule with an improved toxicological profile comprises no ribonucleotides. In one embodiment, a siNA molecule with an improved toxicological profile comprises less than 5 ribonucleotides (e.g., 1, 2, 3, or 4 ribonucleotides). In one embodiment, a siNA molecule with an improved toxicological profile comprises Stab 7, Stab 8, Stab 11, Stab 12, Stab 13, Stab 16, Stab 17, Stab 18, Stab 19, Stab 20, Stab 23, Stab 24, Stab 25, Stab 26, Stab 27, Stab 28, Stab 29, Stab 30, Stab 31, Stab 32 or any combination thereof (see **Table IV**). In one embodiment, the level of immunostimulatory response associated with a given siNA molecule can be measured as is known in the art, for example by determining the level of PKR/interferon response, proliferation, B-cell activation, and/or cytokine production in assays to quantitate the immunostimulatory response of particular siNA molecules (see, for example, Leifer *et al.*, 2003, *J Immunother.* 26, 313-9; and U.S. Patent No. 5968909, incorporated in its entirety by reference).

In one embodiment, the invention features siNA constructs that mediate RNAi against HCV, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the sense and antisense strands of the siNA construct.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the sense and antisense strands of the siNA molecule comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of

step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the sense and antisense strands of the siNA molecule.

In one embodiment, the invention features siNA constructs that mediate RNAi against HCV, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target RNA sequence within a cell.

In one embodiment, the invention features siNA constructs that mediate RNAi against HCV, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target DNA sequence within a cell.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target DNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the antisense strand of the siNA molecule and a complementary target DNA sequence.

In one embodiment, the invention features siNA constructs that mediate RNAi against HCV, wherein the siNA construct comprises one or more chemical modifications described herein that modulate the polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the chemically-modified siNA construct.

In another embodiment, the invention features a method for generating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to a chemically-modified siNA molecule comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the chemically-modified siNA molecule.

In one embodiment, the invention features chemically-modified siNA constructs that mediate RNAi against HCV in a cell, wherein the chemical modifications do not significantly effect the interaction of siNA with a target RNA molecule, DNA molecule and/or proteins or other factors that are essential for RNAi in a manner that would decrease the efficacy of RNAi mediated by such siNA constructs.

In another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against HCV comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity.

In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against HCV target RNA comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the target RNA.

In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against HCV target DNA comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the target DNA.

In one embodiment, the invention features siNA constructs that mediate RNAi against HCV, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the cellular uptake of the siNA construct.

In another embodiment, the invention features a method for generating siNA molecules against HCV with improved cellular uptake comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved cellular uptake.

In one embodiment, the invention features siNA constructs that mediate RNAi against HCV, wherein the siNA construct comprises one or more chemical modifications described herein that increases the bioavailability of the siNA construct, for example, by attaching polymeric conjugates such as polyethyleneglycol or equivalent conjugates that improve the pharmacokinetics of the siNA construct, or by attaching conjugates that target specific tissue types or cell types *in vivo*. Non-limiting examples of such conjugates are described in Vargeese *et al.,* U.S. Serial No. 10/201,394 incorporated by reference herein.

In one embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing a conjugate into the structure of a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such conjugates can include ligands for cellular receptors, such as peptides derived from naturally occurring protein ligands; protein localization sequences, including cellular ZIP code sequences; antibodies; nucleic acid aptamers; vitamins and other co-factors, such as folate and N-acetylgalactosamine; polymers, such as polyethyleneglycol (PEG); phospholipids; cholesterol; polyamines, such as spermine or spermidine; and others.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence is chemically modified in a manner that it can no longer act as a guide sequence for efficiently

mediating RNA interference and/or be recognized by cellular proteins that facilitate RNAi.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein the second sequence is designed or modified in a manner that prevents its entry into the RNAi pathway as a guide sequence or as a sequence that is complementary to a target nucleic acid (e.g., RNA) sequence. Such design or modifications are expected to enhance the activity of siNA and/or improve the specificity of siNA molecules of the invention. These modifications are also expected to minimize any off-target effects and/or associated toxicity.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence is incapable of acting as a guide sequence for mediating RNA interference.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence does not have a terminal 5'-hydroxyl (5'-OH) or 5'-phosphate group.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence comprises a terminal cap moiety at the 5'-end of said second sequence. In one embodiment, the terminal cap moiety comprises an inverted abasic, inverted deoxy abasic, inverted nucleotide moiety, a group shown in **Figure 10**, an alkyl or cycloalkyl group, a heterocycle, or any other group that prevents RNAi activity in which the second sequence serves as a guide sequence or template for RNAi.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence comprises a terminal cap moiety at the 5'-end and 3'-end of said second sequence. In one embodiment, each terminal cap moiety individually comprises an inverted abasic, inverted deoxy abasic, inverted nucleotide moiety, a group shown in **Figure 10**, an alkyl or cycloalkyl group, a heterocycle, or any other group that prevents RNAi activity in which the second sequence serves as a guide sequence or template for RNAi.

In one embodiment, the invention features a method for generating siNA molecules of the invention with improved specificity for down regulating or inhibiting the expression of a target nucleic acid (e.g., a DNA or RNA such as a gene or its corresponding RNA), comprising (a) introducing one or more chemical modifications into the structure of a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved specificity. In another embodiment, the chemical modification used to improve specificity comprises terminal cap modifications at the 5'-end, 3'-end, or both 5' and 3'-ends of the siNA molecule. The terminal cap modifications can comprise, for example, structures shown in **Figure 10** (e.g. inverted deoxyabasic moieties) or any other chemical modification that renders a portion of the siNA molecule (e.g. the sense strand) incapable of mediating RNA interference against an off target nucleic acid sequence. In a non-limiting example, a siNA molecule is designed such that only the antisense sequence of the siNA molecule can serve as a guide sequence for RISC mediated degradation of a corresponding target RNA sequence. This can be accomplished by rendering the sense sequence of the siNA inactive by introducing chemical modifications to the sense strand that preclude recognition of the sense strand as a guide sequence by RNAi machinery. In one embodiment, such chemical modifications comprise any chemical group at the 5'-end of the sense strand of the siNA, or any other group that serves to render the sense strand inactive as a guide sequence for mediating RNA interference. These modifications, for example, can result in a molecule where the 5'-end of the sense strand no longer has a free 5'-hydroxyl (5'-OH) or a free 5'-phosphate group (e.g., phosphate, diphosphate, triphosphate, cyclic phosphate etc.). Non-limiting examples of such siNA constructs are described herein, such as "Stab 9/10", "Stab 7/8", "Stab 7/19", "Stab 17/22", "Stab

23/24", "Stab 24/25", and "Stab 24/26" (e.g., any siNA having Stab 7, 9, 17, 23, or 24 sense strands) chemistries and variants thereof (see **Table IV**) wherein the 5'-end and 3'-end of the sense strand of the siNA do not comprise a hydroxyl group or phosphate group.

In one embodiment, the invention features a method for generating siNA molecules of the invention with improved specificity for down regulating or inhibiting the expression of a target nucleic acid (e.g., a DNA or RNA such as a gene or its corresponding RNA), comprising introducing one or more chemical modifications into the structure of a siNA molecule that prevent a strand or portion of the siNA molecule from acting as a template or guide sequence for RNAi activity. In one embodiment, the inactive strand or sense region of the siNA molecule is the sense strand or sense region of the siNA molecule, i.e. the strand or region of the siNA that does not have complementarity to the target nucleic acid sequence. In one embodiment, such chemical modifications comprise any chemical group at the 5'-end of the sense strand or region of the siNA that does not comprise a 5'-hydroxyl (5'-OH) or 5'-phosphate group, or any other group that serves to render the sense strand or sense region inactive as a guide sequence for mediating RNA interference. Non-limiting examples of such siNA constructs are described herein, such as "Stab 9/10", "Stab 7/8", "Stab 7/19", "Stab 17/22", "Stab 23/24", "Stab 24/25", and "Stab 24/26" (e.g., any siNA having Stab 7, 9, 17, 23, or 24 sense strands) chemistries and variants thereof (see **Table IV**) wherein the 5'-end and 3'-end of the sense strand of the siNA do not comprise a hydroxyl group or phosphate group.

In one embodiment, the invention features a method for screening siNA molecules that are active in mediating RNA interference against a target nucleic acid sequence comprising (a) generating a plurality of unmodified siNA molecules, (b) screening the siNA molecules of step (a) under conditions suitable for isolating siNA molecules that are active in mediating RNA interference against the target nucleic acid sequence, and (c) introducing chemical modifications (e.g. chemical modifications as described herein or as otherwise known in the art) into the active siNA molecules of (b). In one embodiment, the method further comprises re-screening the chemically modified siNA molecules of step (c) under conditions suitable for isolating chemically modified siNA molecules that are active in mediating RNA interference against the target nucleic acid sequence.

In one embodiment, the invention features a method for screening chemically modified siNA molecules that are active in mediating RNA interference against a target nucleic acid sequence comprising (a) generating a plurality of chemically modified siNA molecules (e.g. siNA molecules as described herein or as otherwise known in the art), and (b) screening the siNA molecules of step (a) under conditions suitable for isolating chemically modified siNA molecules that are active in mediating RNA interference against the target nucleic acid sequence.

The term "ligand" refers to any compound or molecule, such as a drug, peptide, hormone, or neurotransmitter, that is capable of interacting with another compound, such as a receptor, either directly or indirectly. The receptor that interacts with a ligand can be present on the surface of a cell or can alternately be an intercellular receptor. Interaction of the ligand with the receptor can result in a biochemical reaction, or can simply be a physical interaction or association.

In another embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing an excipient formulation to a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such excipients include polymers such as cyclodextrins, lipids, cationic lipids, polyamines, phospholipids, nanoparticles, receptors, ligands, and others.

In another embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing nucleotides having any of Formulae I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability.

In another embodiment, polyethylene glycol (PEG) can be covalently attached to siNA compounds of the present invention. The attached PEG can be any molecular weight, preferably from about 100 to about 50,000 daltons (Da).

The present invention can be used alone or as a component of a kit having at least one of the reagents necessary to carry out the *in vitro* or *in vivo* introduction of RNA to test samples and/or subjects. For example, preferred components of the kit include a siNA molecule of the invention and a vehicle that promotes introduction of the siNA into

cells of interest as described herein (e.g., using lipids and other methods of transfection known in the art, see for example Beigelman *et al,* US 6,395,713). The kit can be used for target validation, such as in determining gene function and/or activity, or in drug optimization, and in drug discovery (see for example Usman et al., USSN 60/402,996). Such a kit can also include instructions to allow a user of the kit to practice the invention.

The term "short interfering nucleic acid", "siNA", "short interfering RNA", "siRNA", "short interfering nucleic acid molecule", "short interfering oligonucleotide molecule", or "chemically-modified short interfering nucleic acid molecule" as used herein refers to any nucleic acid molecule capable of inhibiting or down regulating gene expression or viral replication, for example by mediating RNA interference "RNAi" or gene silencing in a sequence-specific manner; see for example Zamore *et al.,* 2000, Cell, 101, 25-33; Bass, 2001, *Nature*, 411, 428-429; Elbashir *et al.,* 2001, *Nature*, 411, 494-498; and Kreutzer *et al.,* International PCT Publication No. WO 00/44895; Zernicka-Goetz *et al.,* International PCT Publication No. WO 01/36646; Fire, International PCT Publication No. WO 99/32619; Plaetinck *et al.,* International PCT Publication No. WO 00/01846; Mello and Fire, International PCT Publication No. WO 01/29058; Deschamps-Depaillette, International PCT Publication No. WO 99/07409; and Li *et al.,* International PCT Publication No. WO 00/44914; Allshire, 2002, *Science,* 297, 1818-1819; Volpe *et al.,* 2002, *Science,* 297, 1833-1837; Jenuwein, 2002, *Science,* 297, 2215-2218; and Hall *et al.,* 2002, *Science,* 297, 2232-2237; Hutvagner and Zamore, 2002, *Science*, 297, 2056-60; McManus *et al.,* 2002, *RNA*, 8, 842-850; Reinhart *et al.,* 2002, *Gene & Dev.,* 16, 1616-1626; and Reinhart & Bartel, 2002, *Science*, 297, 1831). Non limiting examples of siNA molecules of the invention are shown in **Figures 4-6,** and **Tables II and III** herein. For example the siNA can be a double-stranded polynucleotide molecule comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be assembled from two separate oligonucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (i.e. each strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double stranded structure, for

example wherein the double stranded region is about 15 to about 30, *e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 base pairs; the antisense strand comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof (e.g., about 15 to about 25 or more nucleotides of the siNA molecule are complementary to the target nucleic acid or a portion thereof). Alternatively, the siNA is assembled from a single oligonucleotide, where the self-complementary sense and antisense regions of the siNA are linked by means of a nucleic acid based or non-nucleic acid-based linker(s). The siNA can be a polynucleotide with a duplex, asymmetric duplex, hairpin or asymmetric hairpin secondary structure, having self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof, and wherein the circular polynucleotide can be processed either *in vivo* or *in vitro* to generate an active siNA molecule capable of mediating RNAi. The siNA can also comprise a single stranded polynucleotide having nucleotide sequence complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof (for example, where such siNA molecule does not require the presence within the siNA molecule of nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof), wherein the single stranded polynucleotide can further comprise a terminal phosphate group, such as a 5'-phosphate (see for example Martinez *et al.*, 2002, *Cell.*, 110, 563-574 and Schwarz *et al.*, 2002, *Molecular Cell*, 10, 537-568), or 5',3'-diphosphate. In certain embodiments, the siNA molecule of the invention comprises separate sense and antisense sequences or regions, wherein the sense and antisense regions are covalently linked by nucleotide or non-nucleotide linkers molecules as is known in the art, or are alternately non-covalently linked by ionic interactions, hydrogen bonding, van der waals interactions, hydrophobic interactions, and/or stacking interactions. In certain

embodiments, the siNA molecules of the invention comprise nucleotide sequence that is complementary to nucleotide sequence of a target gene. In another embodiment, the siNA molecule of the invention interacts with nucleotide sequence of a target gene in a manner that causes inhibition of expression of the target gene. As used herein, siNA molecules need not be limited to those molecules containing only RNA, but further encompasses chemically-modified nucleotides and non-nucleotides. In certain embodiments, the short interfering nucleic acid molecules of the invention lack 2'-hydroxy (2'-OH) containing nucleotides. Applicant describes in certain embodiments short interfering nucleic acids that do not require the presence of nucleotides having a 2'-hydroxy group for mediating RNAi and as such, short interfering nucleic acid molecules of the invention optionally do not include any ribonucleotides (e.g., nucleotides having a 2'-OH group). Such siNA molecules that do not require the presence of ribonucleotides within the siNA molecule to support RNAi can however have an attached linker or linkers or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups. Optionally, siNA molecules can comprise ribonucleotides at about 5, 10, 20, 30, 40, or 50% of the nucleotide positions. The modified short interfering nucleic acid molecules of the invention can also be referred to as short interfering modified oligonucleotides "siMON." As used herein, the term siNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of mediating sequence specific RNAi, for example short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically-modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term RNAi is meant to be equivalent to other terms used to describe sequence specific RNA interference, such as post transcriptional gene silencing, translational inhibition, or epigenetics. For example, siNA molecules of the invention can be used to epigenetically silence genes at both the post-transcriptional level or the pre-transcriptional level. In a non-limiting example, epigenetic regulation of gene expression by siNA molecules of the invention can result from siNA mediated modification of chromatin structure or methylation pattern to alter gene expression (see, for example, Verdel *et al.,* 2004, *Science,* 303, 672-676; Pal-Bhadra *et al.,* 2004, *Science,* 303, 669-672; Allshire, 2002, *Science,* 297, 1818-1819; Volpe *et al.,* 2002, *Science,* 297, 1833-1837; Jenuwein, 2002, *Science,* 297, 2215-2218; and Hall *et al.,* 2002, *Science,* 297, 2232-2237).

In one embodiment, a siNA molecule of the invention is a duplex forming oligonucleotide "DFO", (see for example **Figures 14-15** and Vaish et al., USSN 10/727,780 filed December 3, 2003 and International PCT Application No. US04/16390, filed May 24, 2004).

In one embodiment, a siNA molecule of the invention is a multifunctional siNA, (see for example **Figures 16-21** and Jadhav *et al.*, USSN 60/543,480 filed February 10, 2004 and International PCT Application No. US04/16390, filed May 24, 2004). In one embodiment, the multifunctional siNA of the invention can comprise sequence targeting, for example, two or more regions of HCV RNA (see for example target sequences in **Tables II and III**). In one embodiment, the multifunctional siNA of the invention can comprise sequence targeting HCV RNA and one or more cellular targets involved in the HCV lifecyle, such as cellular receptors, cell surface molecules, cellular enzymes, cellular transcription factors, and/or cytokines, second messengers, and cellular accessory molecules including, but not limited to, La antigen (see for example Costa-Mattioli *et al.,* 2004, *Mol Cell Biol.,* 24, 6861-70, e.g., Genbank Accession No. NM_003142) (e.g., interferon regulatory factors (IRFs; e.g., Genbank Accession No. AF082503.1); cellular PKR protein kinase (e.g., Genbank Accession No. XM_002661.7); human eukaryotic initiation factors 2B (elF2Bgamma; e.g., Genbank Accession No. AF256223, and/or elF2gamma; e.g., Genbank Accession No. NM_006874.1); human DEAD Box protein (DDX3; e.g., Genbank Accession No. XM_018021.2); and cellular proteins that bind to the poly(U) tract of the HCV 3'-UTR, such as polypyrimidine tract-binding protein (e.g., Genbank Accession Nos. NM_031991.1 and XM_042972.3).

By "asymmetric hairpin" as used herein is meant a linear siNA molecule comprising an antisense region, a loop portion that can comprise nucleotides or non-nucleotides, and a sense region that comprises fewer nucleotides than the antisense region to the extent that the sense region has enough complementary nucleotides to base pair with the antisense region and form a duplex with loop. For example, an asymmetric hairpin siNA molecule of the invention can comprise an antisense region having length sufficient to mediate RNAi in a cell or in vitro system (e.g. about 15 to about 30, or about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides) and a loop region comprising about 4 to about 12 (e.g., about 4, 5, 6, 7, 8, 9, 10, 11, or 12) nucleotides, and a sense region having about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9,

10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides that are complementary to the antisense region. The asymmetric hairpin siNA molecule can also comprise a 5'-terminal phosphate group that can be chemically modified. The loop portion of the asymmetric hairpin siNA molecule can comprise nucleotides, non-nucleotides, linker molecules, or conjugate molecules as described herein.

By "asymmetric duplex" as used herein is meant a siNA molecule having two separate strands comprising a sense region and an antisense region, wherein the sense region comprises fewer nucleotides than the antisense region to the extent that the sense region has enough complementary nucleotides to base pair with the antisense region and form a duplex. For example, an asymmetric duplex siNA molecule of the invention can comprise an antisense region having length sufficient to mediate RNAi in a cell or in vitro system (e.g. about 15 to about 30, or about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides) and a sense region having about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides that are complementary to the antisense region.

By "modulate" is meant that the expression of the gene, or level of RNA molecule or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits is up regulated or down regulated, such that expression, level, or activity is greater than or less than that observed in the absence of the modulator. For example, the term "modulate" can mean "inhibit," but the use of the word "modulate" is not limited to this definition.

By "inhibit", "down-regulate", or "reduce", it is meant that the expression of the gene, or level of RNA molecules or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits, is reduced below that observed in the absence of the nucleic acid molecules (e.g., siNA) of the invention. In one embodiment, inhibition, down-regulation or reduction with an siNA molecule is below that level observed in the presence of an inactive or attenuated molecule. In another embodiment, inhibition, down-regulation, or reduction with siNA molecules is below that level observed in the presence of, for example, an siNA molecule with scrambled sequence or with mismatches. In another embodiment, inhibition, down-regulation, or reduction of gene expression with a nucleic acid molecule of the instant invention is greater in the presence of the nucleic acid molecule than in its

absence. In one embodiment, inhibition, down regulation, or reduction of gene expression is associated with post transcriptional silencing, such as RNAi mediated cleavage of a target nucleic acid molecule (e.g. RNA) or inhibition of translation. In one embodiment, inhibition, down regulation, or reduction of gene expression is associated with pretranscriptional silencing.

By "gene", or "target gene", is meant a nucleic acid that encodes an RNA, for example, nucleic acid sequences including, but not limited to, structural genes encoding a polypeptide. A gene or target gene can also encode a functional RNA (fRNA) or non-coding RNA (ncRNA), such as small temporal RNA (stRNA), micro RNA (miRNA), small nuclear RNA (snRNA), short interfering RNA (siRNA), small nucleolar RNA (snRNA), ribosomal RNA (rRNA), transfer RNA (tRNA) and precursor RNAs thereof. Such non-coding RNAs can serve as target nucleic acid molecules for siNA mediated RNA interference in modulating the activity of fRNA or ncRNA involved in functional or regulatory cellular processes. Abberant fRNA or ncRNA activity leading to disease can therefore be modulated by siNA molecules of the invention. siNA molecules targeting fRNA and ncRNA can also be used to manipulate or alter the genotype or phenotype of a subject, organism or cell, by intervening in cellular processes such as genetic imprinting, transcription, translation, or nucleic acid processing (e.g., transamination, methylation etc.). The target gene can be a gene derived from a cell, an endogenous gene, a transgene, or exogenous genes such as genes of a pathogen, for example a virus, which is present in the cell after infection thereof. The cell containing the target gene can be derived from or contained in any organism, for example a plant, animal, protozoan, virus, bacterium, or fungus. Non-limiting examples of plants include monocots, dicots, or gymnosperms. Non-limiting examples of animals include vertebrates or invertebrates. Non-limiting examples of fungi include molds or yeasts. For a review, see for example Snyder and Gerstein, 2003, *Science,* 300, 258-260.

By "non-canonical base pair" is meant any non-Watson Crick base pair, such as mismatches and/or wobble base pairs, including flipped mismatches, single hydrogen bond mismatches, trans-type mismatches, triple base interactions, and quadruple base interactions. Non-limiting examples of such non-canonical base pairs include, but are not limited to, AC reverse Hoogsteen, AC wobble, AU reverse Hoogsteen, GU wobble, AA N7 amino, CC 2-carbonyl-amino(H1)-N3-amino(H2), GA sheared, UC 4-carbonyl-amino, UU imino-carbonyl, AC reverse wobble, AU Hoogsteen, AU reverse Watson

Crick, CG reverse Watson Crick, GC N3-amino-amino N3, AA N1-amino symmetric, AA N7-amino symmetric, GA N7-N1 amino-carbonyl, GA+ carbonyl-amino N7-N1, GG N1-carbonyl symmetric, GG N3-amino symmetric, CC carbonyl-amino symmetric, CC N3-amino symmetric, UU 2-carbonyl-imino symmetric, UU 4-carbonyl-imino symmetric, AA amino-N3, AA N1-amino, AC amino 2-carbonyl, AC N3-amino, AC N7-amino, AU amino-4-carbonyl, AU N1-imino, AU N3-imino, AU N7-imino, CC carbonyl-amino, GA amino-N1, GA amino-N7, GA carbonyl-amino, GA N3-amino, GC amino-N3, GC carbonyl-amino, GC N3-amino, GC N7-amino, GG amino-N7, GG carbonyl-imino, GG N7-amino, GU amino-2-carbonyl, GU carbonyl-imino, GU imino-2-carbonyl, GU N7-imino, psiU imino-2-carbonyl, UC 4-carbonyl-amino, UC imino-carbonyl, UU imino-4-carbonyl, AC C2-H-N3, GA carbonyl-C2-H, UU imino-4-carbonyl 2 carbonyl-C5-H, AC amino(A) N3(C)-carbonyl, GC imino amino-carbonyl, Gpsi imino-2-carbonyl amino-2- carbonyl, and GU imino amino-2-carbonyl base pairs.

By "HCV" as used herein is meant, any hepatitis C virus or HCV protein, peptide, or polypeptide having HCV activity, such as encoded by HCV Genbank Accession Nos. shown in **Table I**. The term HCV also refers to nucleic acid sequences encoding any HCV protein, peptide, or polypeptide having HCV activity. The term "HCV" is also meant to include other HCV encoding sequence, such as other HCV isoforms, mutant HCV genes, splice variants of HCV genes, and HCV gene polymorphisms. In one embodiment, the term HCV as used herein refers to cellular or host proteins or polynucleotides encoding such proteins or that are otherwise involved in HCV infection and/or replication.

By "homologous sequence" is meant, a nucleotide sequence that is shared by one or more polynucleotide sequences, such as genes, gene transcripts and/or non-coding polynucleotides. For example, a homologous sequence can be a nucleotide sequence that is shared by two or more genes encoding related but different proteins, such as different members of a gene family, different protein epitopes, different protein isoforms or completely divergent genes, such as a cytokine and its corresponding receptors. A homologous sequence can be a nucleotide sequence that is shared by two or more non-coding polynucleotides, such as noncoding DNA or RNA, regulatory sequences, introns, and sites of transcriptional control or regulation. Homologous sequences can also include conserved sequence regions shared by more than one polynucleotide sequence. Homology does not need to be perfect homology (e.g., 100%), as partially homologous

sequences are also contemplated by the instant invention (e.g., 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80% etc.).

By "conserved sequence region" is meant, a nucleotide sequence of one or more regions in a polynucleotide does not vary significantly between generations or from one biological system, subject, or organism to another biological system, subject, or organism. The polynucleotide can include both coding and non-coding DNA and RNA.

By “sense region” is meant a nucleotide sequence of a siNA molecule having complementarity to an antisense region of the siNA molecule. In addition, the sense region of a siNA molecule can comprise a nucleic acid sequence having homology with a target nucleic acid sequence.

By “antisense region” is meant a nucleotide sequence of a siNA molecule having complementarity to a target nucleic acid sequence. In addition, the antisense region of a siNA molecule can optionally comprise a nucleic acid sequence having complementarity to a sense region of the siNA molecule.

By “target nucleic acid” is meant any nucleic acid sequence whose expression or activity is to be modulated. The target nucleic acid can be DNA or RNA.

By "complementarity" is meant that a nucleic acid can form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types. In reference to the nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., RNAi activity. Determination of binding free energies for nucleic acid molecules is well known in the art (see, e.g., Turner *et al.,* 1987, *CSH Symp. Quant. Biol.* LII pp.123-133; Frier *et al.,* 1986, *Proc. Nat. Acad. Sci.* USA 83:9373-9377; Turner *et al.,* 1987, *J. Am. Chem. Soc.* 109:3783-3785). A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule that can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, or 10 nucleotides out of a total of 10 nucleotides in the first oligonucleotide being based paired to a second nucleic acid sequence having 10 nucleotides represents 50%, 60%, 70%, 80%, 90%, and 100% complementary respectively). "Perfectly complementary" means that all the

contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence. In one embodiment, a siNA molecule of the invention comprises about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides that are complementary to one or more target nucleic acid molecules or a portion thereof.

In one embodiment, siNA molecules of the invention that down regulate or reduce HCV gene expression are used for treating, preventing or reducing HCV infection, liver failure, hepatocellular carcinoma, or cirrhosis in a subject or organism.

In one embodiment of the present invention, each sequence of a siNA molecule of the invention is independently about 15 to about 30 nucleotides in length, in specific embodiments about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. In another embodiment, the siNA duplexes of the invention independently comprise about 15 to about 30 base pairs (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30). In another embodiment, one or more strands of the siNA molecule of the invention independently comprises about 15 to about 30 nucleotides (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) that are complementary to a target nucleic acid molecule. In yet another embodiment, siNA molecules of the invention comprising hairpin or circular structures are about 35 to about 55 (*e.g.*, about 35, 40, 45, 50 or 55) nucleotides in length, or about 38 to about 44 (*e.g.*, about 38, 39, 40, 41, 42, 43, or 44) nucleotides in length and comprising about 15 to about 25 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs. Exemplary siNA molecules of the invention are shown in **Table II**. Exemplary synthetic siNA molecules of the invention are shown in **Table III** and/or **Figures 4-5**.

As used herein "cell" is used in its usual biological sense, and does not refer to an entire multicellular organism, e.g., specifically does not refer to a human. The cell can be present in an organism, e.g., birds, plants and mammals such as humans, cows, sheep, apes, monkeys, swine, dogs, and cats. The cell can be prokaryotic (e.g., bacterial cell) or eukaryotic (e.g., mammalian or plant cell). The cell can be of somatic or germ line origin, totipotent or pluripotent, dividing or non-dividing. The cell can also be derived from or can comprise a gamete or embryo, a stem cell, or a fully differentiated cell.

The siNA molecules of the invention are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells or

tissues. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through direct dermal application, transdermal application, or injection, with or without their incorporation in biopolymers. In particular embodiments, the nucleic acid molecules of the invention comprise sequences shown in **Tables II-III** and/or **Figures 4-5**. Examples of such nucleic acid molecules consist essentially of sequences defined in these tables and figures. Furthermore, the chemically modified constructs described in **Table IV** can be applied to any siNA sequence of the invention.

In another aspect, the invention provides mammalian cells containing one or more siNA molecules of this invention. The one or more siNA molecules can independently be targeted to the same or different sites.

By "RNA" is meant a molecule comprising at least one ribonucleotide residue. By "ribonucleotide" is meant a nucleotide with a hydroxyl group at the 2' position of a β-D-ribofuranose moiety. The terms include double-stranded RNA, single-stranded RNA, isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the siNA or internally, for example at one or more nucleotides of the RNA. Nucleotides in the RNA molecules of the instant invention can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as analogs or analogs of naturally-occurring RNA.

By "subject" is meant an organism, which is a donor or recipient of explanted cells or the cells themselves. "Subject" also refers to an organism to which the nucleic acid molecules of the invention can be administered. A subject can be a mammal or mammalian cells, including a human or human cells.

The term "phosphorothioate" as used herein refers to an internucleotide linkage having Formula I, wherein Z and/or W comprise a sulfur atom. Hence, the term phosphorothioate refers to both phosphorothioate and phosphorodithioate internucleotide linkages.

The term “phosphonoacetate” as used herein refers to an internucleotide linkage having Formula I, wherein Z and/or W comprise an acetyl or protected acetyl group.

The term “thiophosphonoacetate” as used herein refers to an internucleotide linkage having Formula I, wherein Z comprises an acetyl or protected acetyl group and W comprises a sulfur atom or alternately W comprises an acetyl or protected acetyl group and Z comprises a sulfur atom.

The term "universal base" as used herein refers to nucleotide base analogs that form base pairs with each of the natural DNA/RNA bases with little discrimination between them. Non-limiting examples of universal bases include C-phenyl, C-naphthyl and other aromatic derivatives, inosine, azole carboxamides, and nitroazole derivatives such as 3-nitropyrrole, 4-nitroindole, 5-nitroindole, and 6-nitroindole as known in the art (see for example Loakes, 2001, *Nucleic Acids Research,* 29, 2437-2447).

The term "acyclic nucleotide" as used herein refers to any nucleotide having an acyclic ribose sugar, for example where any of the ribose carbons (C1, C2, C3, C4, or C5), are independently or in combination absent from the nucleotide.

The nucleic acid molecules of the instant invention, individually, or in combination or in conjunction with other drugs, can be used to treat, inhibit, reduce, or prevent HCV infection, liver failure, hepatocellular carcinoma, and/or cirrhosis in a subject or organism. For example, the siNA molecules can be administered to a subject or can be administered to other appropriate cells evident to those skilled in the art, individually or in combination with one or more drugs under conditions suitable for the treatment.

In a further embodiment, the siNA molecules can be used in combination with other known treatments to treat, inhibit, reduce, or prevent HCV infection, liver failure, hepatocellular carcinoma, and/or cirrhosis in a subject or organism. For example, the described molecules could be used in combination with one or more known compounds, treatments, or procedures to treat, inhibit, reduce, or prevent HCV infection, liver failure, hepatocellular carcinoma, and/or cirrhosis in a subject or organism as are known in the art.

In one embodiment, the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the invention, in a manner

which allows expression of the siNA molecule. For example, the vector can contain sequence(s) encoding both strands of a siNA molecule comprising a duplex. The vector can also contain sequence(s) encoding a single nucleic acid molecule that is self-complementary and thus forms a siNA molecule. Non-limiting examples of such expression vectors are described in Paul *et al.,* 2002, *Nature Biotechnology,* 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology,* 19, 497; Lee *et al.,* 2002, *Nature Biotechnology,* 19, 500; and Novina *et al.,* 2002, *Nature Medicine*, advance online publication doi:10.1038/nm725.

In another embodiment, the invention features a mammalian cell, for example, a human cell, including an expression vector of the invention.

In yet another embodiment, the expression vector of the invention comprises a sequence for a siNA molecule having complementarity to a RNA molecule referred to by a Genbank Accession numbers, for example Genbank Accession Nos. shown in **Table I.**

In one embodiment, an expression vector of the invention comprises a nucleic acid sequence encoding two or more siNA molecules, which can be the same or different.

In another aspect of the invention, siNA molecules that interact with target RNA molecules and down-regulate gene encoding target RNA molecules (for example target RNA molecules referred to by Genbank Accession numbers herein) are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the siNA molecules bind and down-regulate gene function or expression via RNA interference (RNAi). Delivery of siNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired target cell.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a non-limiting example of a scheme for the synthesis of siNA molecules. The complementary siNA sequence strands, strand 1 and strand 2, are synthesized in tandem and are connected by a cleavable linkage, such as a nucleotide succinate or abasic succinate, which can be the same or different from the cleavable linker used for solid phase synthesis on a solid support. The synthesis can be either solid phase or solution phase, in the example shown, the synthesis is a solid phase synthesis. The synthesis is performed such that a protecting group, such as a dimethoxytrityl group, remains intact on the terminal nucleotide of the tandem oligonucleotide. Upon cleavage and deprotection of the oligonucleotide, the two siNA strands spontaneously hybridize to form a siNA duplex, which allows the purification of the duplex by utilizing the properties of the terminal protecting group, for example by applying a trityl on purification method wherein only duplexes/oligonucleotides with the terminal protecting group are isolated.

Figure 2 shows a MALDI-TOF mass spectrum of a purified siNA duplex synthesized by a method of the invention. The two peaks shown correspond to the predicted mass of the separate siNA sequence strands. This result demonstrates that the siNA duplex generated from tandem synthesis can be purified as a single entity using a simple trityl-on purification methodology.

Figure 3 shows a non-limiting proposed mechanistic representation of target RNA degradation involved in RNAi. Double-stranded RNA (dsRNA), which is generated by RNA-dependent RNA polymerase (RdRP) from foreign single-stranded RNA, for example viral, transposon, or other exogenous RNA, activates the DICER enzyme that in turn generates siNA duplexes. Alternately, synthetic or expressed siNA can be introduced directly into a cell by appropriate means. An active siNA complex forms which recognizes a target RNA, resulting in degradation of the target RNA by the RISC endonuclease complex or in the synthesis of additional RNA by RNA-dependent RNA

polymerase (RdRP), which can activate DICER and result in additional siNA molecules, thereby amplifying the RNAi response.

Figure 4A-F shows non-limiting examples of chemically-modified siNA constructs of the present invention. In the figure, N stands for any nucleotide (adenosine, guanosine, cytosine, uridine, or optionally thymidine, for example thymidine can be substituted in the overhanging regions designated by parenthesis (N N). Various modifications are shown for the sense and antisense strands of the siNA constructs.

Figure 4A: The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all nucleotides present are ribonucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all nucleotides present are ribonucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

Figure 4B: The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other

modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the sense and antisense strand.

Figure 4C: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl or 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

Figure 4D: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein and wherein and all purine nucleotides that may be present are 2'-deoxy nucleotides. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

Figure 4E: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as “s”, optionally connects the (N N) nucleotides in the antisense strand.

Figure 4F: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein and wherein and all purine nucleotides that may be present are 2'-deoxy nucleotides. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-deoxy nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as “s”, optionally connects the (N N) nucleotides in the antisense strand. The antisense strand of constructs A-F comprise sequence complementary to any target nucleic acid sequence of the invention. Furthermore, when a glyceryl moiety (L) is present at the 3’-end of the

antisense strand for any construct shown in **Figure 4 A-F**, the modified internucleotide linkage is optional.

Figure 5A-F shows non-limiting examples of specific chemically-modified siNA sequences of the invention. **A-F** applies the chemical modifications described in **Figure 4A-F** to a HCV siNA sequence. Such chemical modifications can be applied to any HCV sequence and/or cellular target sequence.

Figure 6 shows non-limiting examples of different siNA constructs of the invention. The examples shown (constructs 1, 2, and 3) have 19 representative base pairs; however, different embodiments of the invention include any number of base pairs described herein. Bracketed regions represent nucleotide overhangs, for example, comprising about 1, 2, 3, or 4 nucleotides in length, preferably about 2 nucleotides. Constructs 1 and 2 can be used independently for RNAi activity. Construct 2 can comprise a polynucleotide or non-nucleotide linker, which can optionally be designed as a biodegradable linker. In one embodiment, the loop structure shown in construct 2 can comprise a biodegradable linker that results in the formation of construct 1 *in vivo* and/or *in vitro*. In another example, construct 3 can be used to generate construct 2 under the same principle wherein a linker is used to generate the active siNA construct 2 *in vivo* and/or *in vitro*, which can optionally utilize another biodegradable linker to generate the active siNA construct 1 *in vivo* and/or *in vitro*. As such, the stability and/or activity of the siNA constructs can be modulated based on the design of the siNA construct for use *in vivo* or *in vitro* and/or *in vitro*.

Figure 7A-C is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate siNA hairpin constructs.

Figure 7A: A DNA oligomer is synthesized with a 5'-restriction site (R1) sequence followed by a region having sequence identical (sense region of siNA) to a predetermined HCV target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, which is followed by a loop sequence of defined sequence (X), comprising, for example, about 3 to about 10 nucleotides.

Figure 7B: The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self-complementary sequence that will result in a

siNA transcript having specificity for a HCV target sequence and having self-complementary sense and antisense regions.

Figure 7C: The construct is heated (for example to about 95°C) to linearize the sequence, thus allowing extension of a complementary second DNA strand using a primer to the 3'-restriction sequence of the first strand. The double-stranded DNA is then inserted into an appropriate vector for expression in cells. The construct can be designed such that a 3'-terminal nucleotide overhang results from the transcription, for example, by engineering restriction sites and/or utilizing a poly-U termination region as described in Paul *et al.,* 2002, *Nature Biotechnology,* 29, 505-508.

Figure 8A-C is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate double-stranded siNA constructs.

Figure 8A: A DNA oligomer is synthesized with a 5'-restriction (R1) site sequence followed by a region having sequence identical (sense region of siNA) to a predetermined HCV target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, and which is followed by a 3'-restriction site (R2) which is adjacent to a loop sequence of defined sequence (X).

Figure 8B: The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self-complementary sequence.

Figure 8C: The construct is processed by restriction enzymes specific to R1 and R2 to generate a double-stranded DNA which is then inserted into an appropriate vector for expression in cells. The transcription cassette is designed such that a U6 promoter region flanks each side of the dsDNA which generates the separate sense and antisense strands of the siNA. Poly T termination sequences can be added to the constructs to generate U overhangs in the resulting transcript.

Figure 9A-E is a diagrammatic representation of a method used to determine target sites for siNA mediated RNAi within a particular target nucleic acid sequence, such as messenger RNA.

Figure 9A: A pool of siNA oligonucleotides are synthesized wherein the antisense region of the siNA constructs has complementarity to target sites across the target

nucleic acid sequence, and wherein the sense region comprises sequence complementary to the antisense region of the siNA.

Figure 9B&C: (**Figure 9B**) The sequences are pooled and are inserted into vectors such that (**Figure 9C**) transfection of a vector into cells results in the expression of the siNA.

Figure 9D: Cells are sorted based on phenotypic change that is associated with modulation of the target nucleic acid sequence.

Figure 9E: The siNA is isolated from the sorted cells and is sequenced to identify efficacious target sites within the target nucleic acid sequence.

Figure 10 shows non-limiting examples of different stabilization chemistries (1-10) that can be used, for example, to stabilize the 3'-end of siNA sequences of the invention, including **(1)** [3-3']-inverted deoxyribose; **(2)** deoxyribonucleotide; **(3)** [5'-3']-3'-deoxyribonucleotide; **(4)** [5'-3']-ribonucleotide; **(5)** [5'-3']-3'-O-methyl ribonucleotide; **(6)** 3'-glyceryl; **(7)** [3'-5']-3'-deoxyribonucleotide; **(8)** [3'-3']-deoxyribonucleotide; **(9)** [5'-2']-deoxyribonucleotide; and **(10)** [5-3']-dideoxyribonucleotide. In addition to modified and unmodified backbone chemistries indicated in the figure, these chemistries can be combined with different backbone modifications as described herein, for example, backbone modifications having Formula I. In addition, the 2'-deoxy nucleotide shown 5' to the terminal modifications shown can be another modified or unmodified nucleotide or non-nucleotide described herein, for example modifications having any of Formulae I-VII or any combination thereof.

Figure 11 shows a non-limiting example of a strategy used to identify chemically modified siNA constructs of the invention that are nuclease resistance while preserving the ability to mediate RNAi activity. Chemical modifications are introduced into the siNA construct based on educated design parameters (e.g. introducing 2'-mofications, base modifications, backbone modifications, terminal cap modifications etc). The modified construct in tested in an appropriate system (e.g. human serum for nuclease resistance, shown, or an animal model for PK/delivery parameters). In parallel, the siNA construct is tested for RNAi activity, for example in a cell culture system such as a luciferase reporter assay). Lead siNA constructs are then identified which possess a particular characteristic while maintaining RNAi activity, and can be further modified

and assayed once again. This same approach can be used to identify siNA-conjugate molecules with improved pharmacokinetic profiles, delivery, and RNAi activity.

Figure 12 shows non-limiting examples of phosphorylated siNA molecules of the invention, including linear and duplex constructs and asymmetric derivatives thereof.

Figure 13 shows non-limiting examples of chemically modified terminal phosphate groups of the invention.

Figure 14A shows a non-limiting example of methodology used to design self complementary DFO constructs utilizing palindrome and/or repeat nucleic acid sequences that are identified in a target nucleic acid sequence. (i) A palindrome or repeat sequence is identified in a nucleic acid target sequence. (ii) A sequence is designed that is complementary to the target nucleic acid sequence and the palindrome sequence. (iii) An inverse repeat sequence of the non-palindrome/repeat portion of the complementary sequence is appended to the 3'-end of the complementary sequence to generate a self complementary DFO molecule comprising sequence complementary to the nucleic acid target. (iv) The DFO molecule can self-assemble to form a double stranded oligonucleotide. **Figure 14B** shows a non-limiting representative example of a duplex forming oligonucleotide sequence. **Figure 14C** shows a non-limiting example of the self assembly schematic of a representative duplex forming oligonucleotide sequence. **Figure 14D** shows a non-limiting example of the self assembly schematic of a representative duplex forming oligonucleotide sequence followed by interaction with a target nucleic acid sequence resulting in modulation of gene expression.

Figure 15 shows a non-limiting example of the design of self complementary DFO constructs utilizing palindrome and/or repeat nucleic acid sequences that are incorporated into the DFO constructs that have sequence complementary to any target nucleic acid sequence of interest. Incorporation of these palindrome/repeat sequences allow the design of DFO constructs that form duplexes in which each strand is capable of mediating modulation of target gene expression, for example by RNAi. First, the target sequence is identified. A complementary sequence is then generated in which nucleotide or non-nucleotide modifications (shown as X or Y) are introduced into the complementary sequence that generate an artificial palindrome (shown as XYXYXY in the Figure). An inverse repeat of the non-palindrome/repeat complementary sequence is appended to the 3'-end of the complementary sequence to generate a self complementary

DFO comprising sequence complementary to the nucleic acid target. The DFO can self-assemble to form a double stranded oligonucleotide.

Figure 16 shows non-limiting examples of multifunctional siNA molecules of the invention comprising two separate polynucleotide sequences that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences. **Figure 16A** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 3'-ends of each polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. **Figure 16B** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 5'-ends of each polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences.

Figure 17 shows non-limiting examples of multifunctional siNA molecules of the invention comprising a single polynucleotide sequence comprising distinct regions that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences. **Figure 17A** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the second complementary region is situated at the 3'-end of the polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. **Figure 17B** shows a non-limiting example of a multifunctional siNA

molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first complementary region is situated at the 5'-end of the polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. In one embodiment, these multifunctional siNA constructs are processed in vivo or in vitro to generate multifunctional siNA constructs as shown in **Figure 16.**

Figure 18 shows non-limiting examples of multifunctional siNA molecules of the invention comprising two separate polynucleotide sequences that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences and wherein the multifunctional siNA construct further comprises a self complementary, palindrome, or repeat region, thus enabling shorter bifuctional siNA constructs that can mediate RNA interference against differing target nucleic acid sequences. **Figure 18A** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 3'-ends of each polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. **Figure 18B** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 5'-ends of each polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional

siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences.

Figure 19 shows non-limiting examples of multifunctional siNA molecules of the invention comprising a single polynucleotide sequence comprising distinct regions that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences and wherein the multifunctional siNA construct further comprises a self complementary, palindrome, or repeat region, thus enabling shorter bifuctional siNA constructs that can mediate RNA interference against differing target nucleic acid sequences. **Figure 19A** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the second complementary region is situated at the 3'-end of the polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. **Figure 19B** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first complementary region is situated at the 5'-end of the polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. In one embodiment, these multifunctional siNA constructs are processed in vivo or in vitro to generate multifunctional siNA constructs as shown in **Figure 18.**

Figure 20 shows a non-limiting example of how multifunctional siNA molecules of the invention can target two separate target nucleic acid molecules, such as separate RNA molecules encoding differing proteins, for example, a cytokine and its corresponding receptor, differing viral strains, a virus and a cellular protein involved in

viral infection or replication, or differing proteins involved in a common or divergent biologic pathway that is implicated in the maintenance of progression of disease. Each strand of the multifunctional siNA construct comprises a region having complementarity to separate target nucleic acid molecules. The multifunctional siNA molecule is designed such that each strand of the siNA can be utilized by the RISC complex to initiate RNA interference mediated cleavage of its corresponding target. These design parameters can include destabilization of each end of the siNA construct (see for example Schwarz *et al.,* 2003, *Cell,* 115, 199-208). Such destabilization can be accomplished for example by using guanosine-cytidine base pairs, alternate base pairs (e.g., wobbles), or destabilizing chemically modified nucleotides at terminal nucleotide positions as is known in the art.

Figure 21 shows a non-limiting example of how multifunctional siNA molecules of the invention can target two separate target nucleic acid sequences within the same target nucleic acid molecule, such as alternate coding regions of a RNA, coding and non-coding regions of a RNA, or alternate splice variant regions of a RNA. Each strand of the multifunctional siNA construct comprises a region having complementarity to the separate regions of the target nucleic acid molecule. The multifunctional siNA molecule is designed such that each strand of the siNA can be utilized by the RISC complex to initiate RNA interference mediated cleavage of its corresponding target region. These design parameters can include destabilization of each end of the siNA construct (see for example Schwarz *et al.,* 2003, *Cell,* 115, 199-208). Such destabilization can be accomplished for example by using guanosine-cytidine base pairs, alternate base pairs (e.g., wobbles), or destabilizing chemically modified nucleotides at terminal nucleotide positions as is known in the art.

Figure 22 shows non-limiting examples of inhibition of viral replication of a HCV/poliovirus chimera by siNA constructs targeted to HCV chimera (29579/29586; 29578/29585) compared to control (29593/29600).

Figure 23 shows a non-limiting example of a dose response study demonstrating the inhibition of viral replication of a HCV/poliovirus chimera by siNA construct (29579/29586) at various concentrations (1nM, 5nM, 10nM, and 25nM) compared to control (29593/29600).

Figure 24 shows a non-limiting example demonstrating the inhibition of viral replication of a HCV/poliovirus chimera by a chemically modified siRNA construct (30051/30053) compared to control construct (30052/30054).

Figure 25 shows a non-limiting example demonstrating the inhibition of viral replication of a HCV/poliovirus chimera by a chemically modified siRNA construct (30055/30057) compared to control construct (30056/30058).

Figure 26 shows a non-limiting example of several chemically modified siRNA constructs targeting viral replication of an HCV/poliovirus chimera at 10 nM treatment in comparison to a lipid control and an inverse siNA control construct 29593/ 29600.

Figure 27 shows a non-limiting example of several chemically modified siRNA constructs targeting viral replication of a HCV/poliovirus chimera at 25 nM treatment in comparison to a lipid control and an inverse siNA control construct 29593/ 29600.

Figure 28 shows a non-limiting example of several chemically modified siRNA constructs targeting viral replication of a Huh7 HCV replicon system at 25 nM treatment in comparison to untreated cells ("cells"), cells transfected with lipofectamine ("LFA2K") and inverse siNA control constructs ("Inv").

Figure 29 shows a non-limiting example of a dose response study using chemically modified siNA molecules (Stab 4/5, see **Table IV**) targeting HCV RNA sites 291, 300, and 303 in a Huh7 HCV replicon system at 5, 10, 25, and 100 nM treatment in comparison to untreated cells ("cells"), cells transfected with lipofectamine ("LFA") and inverse siNA control constructs ("Inv").

Figure 30 shows a non-limiting example of several chemically modified siNA constructs (Stab 7/8, see **Table IV**) targeting viral replication in a Huh7 HCV replicon system at 25 nM treatment in comparison to untreated cells ("cells"), cells transfected with lipofectamine ("Lipid") and inverse siNA control constructs ("Inv").

Figure 31 shows a non-limiting example of a dose response study using chemically modified siNA molecules (Stab 7/8, see **Table IV**) targeting HCV site 327 in a Huh7 HCV replicon system at 5, 10, 25, 50, and 100 nM treatment in comparison to inverse siNA control constructs.

Figure 32 shows the results of a study in which siNA/interferon combination treatments were assayed using 0-100 nM siNA in a HCV Subgenomic Replicon system in Huh7 cells compared to interferon alone.

Figure 33 shows the results of a dose response study in which multifunctional siNAs targeting sites 304 and 327 (MF 36447/34588/38310) and sites 282 and 304 (MF 34588/36445/38311) of HCV RNA were evaluated with an irrelevant multifunctional siNA control (MF Control) and pools of individual siNA constructs targeting sites 304 (34583/34588) and 327 (34585/32201), and sites 282 (34581/34586) and 304 (34583/34588), along with untreated cells and a transfection control (LFA) from 0.1 to 10 nM. Compound numbers for the siNA constructs are shown in **Table III**. As shown in the figure, the multifunctional siNA constructs show equivalent activity to the corresponding pooled siNA constructs.

Figure 34 shows the results of a dose response study in which chemically stabilized multifunctional siNAs targeting sites 282 and 304 (MF 38314/38294/38300) of HCV RNA was evaluated with individual siNA constructs targeting sites 282 (33139/38294) and 304 (33149/38300) and a pool of the individual siNA constructs targeting sites 282 (33139/38294) and 304 (33149/38300), along with untreated cells and a transfection control (LFA) from 0.1 to 25 nM. Compound numbers for the siNA constructs are shown in **Table III**. As shown in the figure, the multifunctional siNA constructs show equivalent activity to the corresponding individual and pooled siNA constructs.

Figure 35 shows the results of a dose response study in which chemically stabilized multifunctional siNAs targeting sites 282 and 304 (MF 38314/38297/38300) of HCV RNA was evaluated with individual siNA constructs targeting site 282 (33139/38297) and a pool of individual siNA constructs targeting sites 282 (33139/38297) and 304 (33149/38300), along with untreated cells and a transfection control (LFA) from 0.1 to 25 nM. Compound numbers for the siNA constructs are shown in **Table III**. As shown in the figure, the multifunctional siNA constructs show equivalent activity to the corresponding individual and pooled siNA constructs.

Figure 36 shows the results of a dose response study in which chemically stabilized multifunctional siNAs targeting sites 327 and 304 (MF 38312/37791/38300) of HCV RNA was evaluated with individual siNA constructs targeting site 327

(31703/37791) and a pool of individual siNA constructs targeting sites 327 (31703/37791) and 304 (33149/38300), along with untreated cells and a transfection control (LFA) from 0.1 to 25 nM. Compound numbers for the siNA constructs are shown in **Table III**. As shown in the figure, the multifunctional siNA constructs show equivalent activity to the corresponding individual and pooled siNA constructs.

Figure 37 shows the results of a dose response study in which chemically stabilized multifunctional siNAs targeting sites 327 and 304 (MF 38312/38302/38300) of HCV RNA was evaluated with individual siNA constructs targeting site 327 (31703/38302) and a pool of individual siNA constructs targeting sites 327 (31703/38302) and 304 (33149/38300), along with untreated cells and a transfection control (LFA) from 0.1 to 25 nM. Compound numbers for the siNA constructs are shown in **Table III**. As shown in the figure, the multifunctional siNA constructs show equivalent activity to the corresponding individual and pooled siNA constructs.

Figure 38 shows the results of a dose response study in which chemically stabilized multifunctional siNAs targeting sites 327 and 282 (MF 38313/38302/38297) of HCV RNA was evaluated a pool of individual siNA constructs targeting sites 327 (31703/37791) and 282 (33139/38294), and another pool of individual siNA constructs targeting sites 327 (31703/38302) and 282 (33139/38397) along with untreated cells and a transfection control (LFA) from 0.1 to 25 nM. Compound numbers for the siNA constructs are shown in **Table III**. As shown in the figure, the multifunctional siNA constructs show equivalent activity to the corresponding pooled siNA constructs.

Figure 39 shows the results of a dose response study in which chemically stabilized multifunctional siNAs targeting sites 282 and 304 (MF 38314/38297/38300) of HCV RNA was evaluated a pool of individual siNA constructs targeting sites 282 (33139/38294) and 304 (33149/38300), and another pool of individual siNA constructs targeting sites 282 (33139/38297) and 304 (33149/38300) along with untreated cells and a transfection control (LFA) from 0.1 to 25 nM. Compound numbers for the siNA constructs are shown in **Table III**. As shown in the figure, the multifunctional siNA constructs show equivalent activity to the corresponding pooled siNA constructs.

Figure 40 shows the results of a dose response study in which chemically stabilized multifunctional siNAs targeting sites 327 and 304 (MF 38312/38302/38300) of HCV RNA was evaluated a pool of individual siNA constructs targeting sites 327

(31703/37791) and 304 (33149/38300), and another pool of individual siNA constructs targeting sites 327 (31703/38302) and 304 (33149/38300) along with untreated cells and a transfection control (LFA) from 0.1 to 25 nM. Compound numbers for the siNA constructs are shown in **Table III**. As shown in the figure, the multifunctional siNA constructs show equivalent activity to the corresponding pooled siNA constructs.

Figure 41 shows the results of a dose response study in which chemically stabilized multifunctional siNAs targeting sites 282 and 327 (MF 38313/38297/38302) of HCV RNA was evaluated a pool of individual siNA constructs targeting sites 282 (33139/38294) and 327 (31703/37791), and another pool of individual siNA constructs targeting sites 282 (33139/38297) and 327 (31703/38302) along with untreated cells and a transfection control (LFA) from 0.1 to 25 nM. Compound numbers for the siNA constructs are shown in **Table III**. As shown in the figure, the multifunctional siNA constructs show equivalent activity to the corresponding pooled siNA constructs.

Figures 42A-42H shows non-limiting examples of tethered multiifunctional siNA constructs of the invention. In the examples shown, a linker (e.g., nucleotide or non-nucleotide linker) connects two siNA regions (e.g., two sense, two antisense, or alternately a sense and an antisense region together. Separate sense (or sense and antisense) sequences corresponding to a first target sequence and second target sequence are hybridized to their corresponding sense and/or antisense sequences in the multifunctional siNA. In addition, various conjugates, ligands, aptamers, polymers or reporter molecules can be attached to the linker region for selective or improved delivery and/or pharmacokinetic properties.

Figure 43 shows a non-limiting example of various dendrimer based multifunctional siNA designs.

Figure 44 shows a non-limiting example of various supramolecular multifunctional siNA designs.

Figure 45 shows a non-limiting example of a dicer enabled multifunctional siNA design using a 30 nucleotide precursor siNA construct. A 30 base pair duplex is cleaved by Dicer into 22 and 8 base pair products from either end (8 b.p. fragments not shown). For ease of presentation the overhangs generated by dicer are not shown – but can be compensated for. Three targeting sequences are shown. The required sequence identity

overlapped is indicated by grey boxes. The N's of the parent 30 b.p. siNA are suggested sites of 2'-OH positions to enable Dicer cleavage if this is tested in stabilized chemistries. Note that processing of a 30mer duplex by Dicer RNase III does not give a precise 22+8 cleavage, but rather produces a series of closely related products (with 22+8 being the primary site). Therefore, processing by Dicer will yield a series of active siNAs.

Figure 46 shows a non-limiting example of a dicer enabled multifunctional siNA design using a 40 nucleotide precursor siNA construct. A 40 base pair duplex is cleaved by Dicer into 20 base pair products from either end. For ease of presentation the overhangs generated by dicer are not shown – but can be compensated for. Four targeting sequences are shown. The required sequence identity overlapped is indicated by grey boxes. This design format can be extended to larger RNAs. If chemically stabilized siNAs are bound by Dicer, then strategically located ribonucleotide linkages can enable designer cleavage products that permit our more extensive repertoire of multiifunctional designs. For example cleavage products not limited to the Dicer standard of approximately 22-nucleotides can allow multifunctional siNA constructs with a target sequence identity overlap ranging from, for example, about 3 to about 15 nucleotides.

Figure 47 shows a non-limiting example of inhibition of HBV RNA by dicer enabled multifunctional siNA constructs targeting HBV site 263. When the first 17 nucleotides of a siNA antisense strand (e.g., 21 nucleotide strands in a duplex with 3'-TT overhangs) are complementary to a target RNA, robust silencing was observed at 25 nM. 80% silencing was observed with only 16 nucleotide complementarity in the same format.

Figure 48 shows a non-limiting example of additional multifunctional siNA construct designs of the invention. In one example, a conjugate, ligand, aptamer, label, or other moiety is attached to a region of the multifunctional siNA to enable improved delivery or pharmacokinetic profiling.

Figure 49 shows a non-limiting example of additional multifunctional siNA construct designs of the invention. In one example, a conjugate, ligand, aptamer, label, or other moiety is attached to a region of the multifunctional siNA to enable improved delivery or pharmacokinetic profiling.

DETAILED DESCRIPTION OF THE INVENTION

Mechanism of Action of Nucleic Acid Molecules of the Invention

The discussion that follows discusses the proposed mechanism of RNA interference mediated by short interfering RNA as is presently known, and is not meant to be limiting and is not an admission of prior art. Applicant demonstrates herein that chemically-modified short interfering nucleic acids possess similar or improved capacity to mediate RNAi as do siRNA molecules and are expected to possess improved stability and activity *in vivo*; therefore, this discussion is not meant to be limiting only to siRNA and can be applied to siNA as a whole. By "improved capacity to mediate RNAi" or "improved RNAi activity" is meant to include RNAi activity measured *in vitro* and/or *in vivo* where the RNAi activity is a reflection of both the ability of the siNA to mediate RNAi and the stability of the siNAs of the invention. In this invention, the product of these activities can be increased *in vitro* and/or *in vivo* compared to an all RNA siRNA or a siNA containing a plurality of ribonucleotides. In some cases, the activity or stability of the siNA molecule can be decreased (i.e., less than ten-fold), but the overall activity of the siNA molecule is enhanced *in vitro* and/or *in vivo*.

RNA interference refers to the process of sequence specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Fire *et al.,* 1998, *Nature,* 391, 806). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes which is commonly shared by diverse flora and phyla (Fire *et al.,* 1999, *Trends Genet.,* 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response though a mechanism that has yet to be fully characterized. This mechanism appears to be different from the interferon response that results from dsRNA-mediated activation of

protein kinase PKR and 2', 5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as Dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Berstein *et al.*, 2001, *Nature,* 409, 363). Short interfering RNAs derived from Dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes. Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner *et al.*, 2001, *Science,* 293, 834). The RNAi response also features an endonuclease complex containing a siRNA, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence homologous to the siRNA. Cleavage of the target RNA takes place in the middle of the region complementary to the guide sequence of the siRNA duplex (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188). In addition, RNA interference can also involve small RNA (e.g., micro-RNA or miRNA) mediated gene silencing, presumably though cellular mechanisms that regulate chromatin structure and thereby prevent transcription of target gene sequences (see for example Allshire, 2002, *Science,* 297, 1818-1819; Volpe *et al.*, 2002, *Science,* 297, 1833-1837; Jenuwein, 2002, *Science,* 297, 2215-2218; and Hall *et al.*, 2002, *Science,* 297, 2232-2237). As such, siNA molecules of the invention can be used to mediate gene silencing via interaction with RNA transcripts or alternately by interaction with particular gene sequences, wherein such interaction results in gene silencing either at the transcriptional level or post-transcriptional level.

RNAi has been studied in a variety of systems. Fire *et al.*, 1998, *Nature,* 391, 806, were the first to observe RNAi in *C. elegans*. Wianny and Goetz, 1999, *Nature Cell Biol.*, 2, 70, describe RNAi mediated by dsRNA in mouse embryos. Hammond *et al.*, 2000, *Nature,* 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir *et al.*, 2001, *Nature,* 411, 494, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in Drosophila embryonic lysates has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown

that 21 nucleotide siRNA duplexes are most active when containing two 2-nucleotide 3'-terminal nucleotide overhangs. Furthermore, substitution of one or both siRNA strands with 2'-deoxy or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of 3'-terminal siRNA nucleotides with deoxy nucleotides was shown to be tolerated. Mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen *et al.*, 2001, *Cell,* 107, 309); however, siRNA molecules lacking a 5'-phosphate are active when introduced exogenously, suggesting that 5'-phosphorylation of siRNA constructs may occur *in vivo*.

Duplex Foming Oligonucleotides (DFO) of the Invention

In one embodiment, the invention features siNA molecules comprising duplex forming oligonucleotides (DFO) that can self-assemble into double stranded oligonucleotides. The duplex forming oligonucleotides of the invention can be chemically synthesized or expressed from transcription units and/or vectors. The DFO molecules of the instant invention provide useful reagents and methods for a variety of therapeutic, diagnostic, agricultural, veterinary, target validation, genomic discovery, genetic engineering and pharmacogenomic applications.

Applicant demonstrates herein that certain oligonucleotides, refered to herein for convenience but not limitation as duplex forming oligonucleotides or DFO molecules, are potent mediators of sequence specific regulation of gene expression. The oligonucleotides of the invention are distinct from other nucleic acid sequences known in the art (e.g., siRNA, miRNA, stRNA, shRNA, antisense oligonucleotides etc.) in that they represent a class of linear polynucleotide sequences that are designed to self-assemble into double stranded oligonucleotides, where each strand in the double stranded oligonucleotides comprises a nucleotide sequence that is complementary to a target nucleic acid molecule. Nucleic acid molecules of the invention can thus self assemble into functional duplexes in which each strand of the duplex comprises the same

polynucleotide sequence and each strand comprises a nucleotide sequence that is complementary to a target nucleic acid molecule.

Generally, double stranded oligonucleotides are formed by the assembly of two distinct oligonucleotide sequences where the oligonucleotide sequence of one strand is complementary to the oligonucleotide sequence of the second strand; such double stranded oligonucleotides are assembled from two separate oligonucleotides, or from a single molecule that folds on itself to form a double stranded structure, often referred to in the field as hairpin stem-loop structure (e.g., shRNA or short hairpin RNA). These double stranded oligonucleotides known in the art all have a common feature in that each strand of the duplex has a distict nucleotide sequence.

Distinct from the double stranded nucleic acid molecules known in the art, the applicants have developed a novel, potentially cost effective and simplified method of forming a double stranded nucleic acid molecule starting from a single stranded or linear oligonucleotide. The two strands of the double stranded oligonucleotide formed according to the instant invention have the same nucleotide sequence and are not covalently linked to each other. Such double-stranded oligonucleotides molecules can be readily linked post-synthetically by methods and reagents known in the art and are within the scope of the invention. In one embodiment, the single stranded oligonucleotide of the invention (the duplex forming oligonucleotide) that forms a double stranded oligonucleotide comprises a first region and a second region, where the second region includes a nucleotide sequence that is an inverted repeat of the nucleotide sequence in the first region, or a portion thereof, such that the single stranded oligonucleotide self assembles to form a duplex oligonucleotide in which the nucleotide sequence of one strand of the duplex is the same as the nucleotide sequence of the second strand. Non-limiting examples of such duplex forming oligonucleotides are illustrated in **Figures 14 and 15**. These duplex forming oligonucleotides (DFOs) can optionally include certain palindrome or repeat sequences where such palindrome or repeat sequences are present in between the first region and the second region of the DFO.

In one embodiment, the invention features a duplex forming oligonucleotide (DFO) molecule, wherein the DFO comprises a duplex forming self complementary nucleic acid sequence that has nucleotide sequence complementary to a HCV target

nucleic acid sequence. The DFO molecule can comprise a single self complementary sequence or a duplex resulting from assembly of such self complementary sequences.

In one embodiment, a duplex forming oligonucleotide (DFO) of the invention comprises a first region and a second region, wherein the second region comprises a nucleotide sequence comprising an inverted repeat of nucleotide sequence of the first region such that the DFO molecule can assemble into a double stranded oligonucleotide. Such double stranded oligonucleotides can act as a short interfering nucleic acid (siNA) to modulate gene expression. Each strand of the double stranded oligonucleotide duplex formed by DFO molecules of the invention can comprise a nucleotide sequence region that is complementary to the same nucleotide sequence in a target nucleic acid molecule (e.g., target HCV RNA).

In one embodiment, the invention features a single stranded DFO that can assemble into a double stranded oligonucleotide. The applicant has surprisingly found that a single stranded oligonucleotide with nucleotide regions of self complementarity can readily assemble into duplex oligonucleotide constructs. Such DFOs can assemble into duplexes that can inhibit gene expression in a sequence specific manner. The DFO moleucles of the invention comprise a first region with nucleotide sequence that is complementary to the nucleotide sequence of a second region and where the sequence of the first region is complementary to a target nucleic acid (e.g., RNA). The DFO can form a double stranded oligonucleotide wherein a portion of each strand of the double stranded oligonucleotide comprises a sequence complementary to a target nucleic acid sequence.

In one embodiment, the invention features a double stranded oligonucleotide, wherein the two strands of the double stranded oligonucleotide are not covalently linked to each other, and wherein each strand of the double stranded oligonucleotide comprises a nucleotide sequence that is complementary to the same nucleotide sequence in a target nucleic acid molecule or a portion thereof (e.g., HCV RNA target). In another embodiment, the two strands of the double stranded oligonucleotide share an identical nucleotide sequence of at least about 15, preferably at least about 16, 17, 18, 19, 20, or 21 nucleotides.

In one embodiment, a DFO molecule of the invention comprises a structure having Formula DFO-I:

$$5'\text{-p-X Z X'-3'}$$

wherein Z comprises a palindromic or repeat nucleic acid sequence optionally with one or more modified nucleotides (e.g., nucleotide with a modified base, such as 2-amino purine, 2-amino-1,6-dihydro purine or a universal base), for example of length about 2 to about 24 nucleotides in even numbers (e.g., about 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, or 22 or 24 nucleotides), X represents a nucleic acid sequence, for example of length between about 1 to about 21 nucleotides (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 nucleotides), X' comprises a nucleic acid sequence, for example of length about 1 and about 21 nucleotides (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 nucleotides) having nucleotide sequence complementarity to sequence X or a portion thereof, p comprises a terminal phosphate group that can be present or absent, and wherein sequence X and Z, either independently or together, comprise nucleotide sequence that is complementary to a target nucleic acid sequence or a portion thereof and is of length sufficient to interact (e.g., base pair) with the target nucleic acid sequence or a portion thereof (e.g., HCV RNA target). For example, X independently can comprise a sequence from about 12 to about 21 or more (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more) nucleotides in length that is complementary to nucleotide sequence in a target HCV RNA or a portion thereof. In another non-limiting example, the length of the nucleotide sequence of X and Z together, when X is present, that is complementary to the target RNA or a portion thereof (e.g., HCV RNA target) is from about 12 to about 21 or more nucleotides (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more). In yet another non-limiting example, when X is absent, the length of the nucleotide sequence of Z that is complementary to the target HCV RNA or a portion thereof is from about 12 to about 24 or more nucleotides (e.g., about 12, 14, 16, 18, 20, 22, 24, or more). In one embodiment X, Z and X' are independently oligonucleotides, where X and/or Z comprises a nucleotide sequence of length sufficient to interact (e.g., base pair) with a nucleotide sequence in the target RNA or a portion thereof (e.g., HCV RNA target). In one embodiment, the lengths of oligonucleotides X and X' are identical. In another embodiment, the lengths of oligonucleotides X and X' are not identical. In another embodiment, the lengths of oligonucleotides X and Z, or Z and X', or X, Z and X' are either identical or different.

When a sequence is described in this specification as being of "sufficient" length to interact (*i.e.*, base pair) with another sequence, it is meant that the the length is such that the number of bonds (*e.g.*, hydrogen bonds) formed between the two sequences is enough to enable the two sequence to form a duplex under the conditions of interest. Such conditions can be *in vitro* (*e.g.*, for diagnostic or assay purposes) or *in vivo* (*e.g.*, for therapeutic purposes). It is a simple and routine matter to determine such lengths.

In one embodiment, the invention features a double stranded oligonucleotide construct having Formula DFO-I(a):

5'-p-X Z X'-3'
3'-X' Z X-p-5'

wherein Z comprises a palindromic or repeat nucleic acid sequence or palindromic or repeat-like nucleic acid sequence with one or more modified nucleotides (e.g., nucleotides with a modified base, such as 2-amino purine, 2-amino-1,6-dihydro purine or a universal base), for example of length about 2 to about 24 nucleotides in even numbers (e.g., about 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 24 nucleotides), X represents a nucleic acid sequence, for example of length about 1 to about 21 nucleotides (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 nucleotides), X' comprises a nucleic acid sequence, for example of length about 1 to about 21 nucleotides (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 nucleotides) having nucleotide sequence complementarity to sequence X or a portion thereof, p comprises a terminal phosphate group that can be present or absent, and wherein each X and Z independently comprises a nucleotide sequence that is complementary to a target nucleic acid sequence or a portion thereof (e.g., HCV RNA target) and is of length sufficient to interact with the target nucleic acid sequence of a portion thereof (e.g., HCV RNA target). For example, sequence X independently can comprise a sequence from about 12 to about 21 or more nucleotides (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more) in length that is complementary to a nucleotide sequence in a target RNA or a portion thereof (e.g., HCV RNA target). In another non-limiting example, the length of the nucleotide sequence of X and Z together (when X is present) that is complementary to the target HCV RNA or a portion thereof is from about 12 to about 21 or more nucleotides (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more). In yet another non-limiting example, when X is absent, the length of the nucleotide sequence of Z that

is complementary to the target HCV RNA or a portion thereof is from about 12 to about 24 or more nucleotides (e.g., about 12, 14, 16, 18, 20, 22, 24 or more). In one embodiment X, Z and X' are independently oligonucleotides, where X and/or Z comprises a nucleotide sequence of length sufficient to interact (e.g., base pair) with nucleotide sequence in the target RNA or a portion thereof (e.g., HCV RNA target). In one embodiment, the lengths of oligonucleotides X and X' are identical. In another embodiment, the lengths of oligonucleotides X and X' are not identical. In another embodiment, the lengths of oligonucleotides X and Z or Z and X' or X, Z and X' are either identical or different. In one embodiment, the double stranded oligonucleotide construct of Formula I(a) includes one or more, specifically 1, 2, 3 or 4, mismatches, to the extent such mismatches do not significantly diminish the ability of the double stranded oligonucleotide to inhibit target gene expression.

In one embodiment, a DFO molecule of the invention comprises structure having Formula DFO-II:

$$5'\text{-p-X X'-}3'$$

wherein each X and X' are independently oligonucleotides of length about 12 nucleotides to about 21 nucleotides, wherein X comprises, for example, a nucleic acid sequence of length about 12 to about 21 nucleotides (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 nucleotides), X' comprises a nucleic acid sequence, for example of length about 12 to about 21 nucleotides (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 nucleotides) having nucleotide sequence complementarity to sequence X or a portion thereof, p comprises a terminal phosphate group that can be present or absent, and wherein X comprises a nucleotide sequence that is complementary to a target nucleic acid sequence (e.g., HCV RNA) or a portion thereof and is of length sufficient to interact (e.g., base pair) with the target nucleic acid sequence of a portion thereof. In one embodiment, the length of oligonucleotides X and X' are identical. In another embodiment the length of oligonucleotides X and X' are not identical. In one embodiment, length of the oligonucleotides X and X' are sufficint to form a relatively stable double stranded oligonucleotide.

In one embodiment, the invention features a double stranded oligonucleotide construct having Formula DFO-II(a):

$$\begin{array}{l} 5'\text{-p-X X'-3'} \\ \quad 3'\text{-X' X-p-5'} \end{array}$$

wherein each X and X' are independently oligonucleotides of length about 12 nucleotides to about 21 nucleotides, wherein X comprises a nucleic acid sequence, for example of length about 12 to about 21 nucleotides (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 nucleotides), X' comprises a nucleic acid sequence, for example of length about 12 to about 21 nucleotides (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 nucleotides) having nucleotide sequence complementarity to sequence X or a portion thereof, p comprises a terminal phosphate group that can be present or absent, and wherein X comprises nucleotide sequence that is complementary to a target nucleic acid sequence or a portion thereof (e.g., HCV RNA target) and is of length sufficient to interact (e.g., base pair) with the target nucleic acid sequence (e.g., HCV RNA) or a portion thereof. In one embodiment, the lengths of oligonucleotides X and X' are identical. In another embodiment, the lengths of oligonucleotides X and X' are not identical. In one embodiment, the lengths of the oligonucleotides X and X' are sufficint to form a relatively stable double stranded oligonucleotide. In one embodiment, the double stranded oligonucleotide construct of Formula II(a) includes one or more, specifically 1, 2, 3 or 4 , mismatches, to the extent such mismatches do not significantly diminish the ability of the double stranded oligonucleotide to inhibit target gene expression.

In one embodiment, the invention features a DFO molecule having Formula DFO-I(b):

$$5'\text{-p-Z-3'}$$

where Z comprises a palindromic or repeat nucleic acid sequence optionally including one or more non-standard or modified nucleotides (e.g., nucleotide with a modified base, such as 2-amino purine or a universal base) that can facilitate base-pairing with other nucleotides. Z can be, for example, of length sufficient to interact (e.g., base pair) with nucleotide sequence of a target nucleic acid (e.g., HCV RNA) molecule, preferably of length of at least 12 nucleotides, specifically about 12 to about 24 nucleotides (e.g., about 12, 14, 16, 18, 20, 22 or 24 nucleotides). p represents a terminal phosphate group that can be present or absent.

In one embodiment, a DFO molecule having any of Formula DFO-I, DFO-I(a), DFO-I(b), DFO-II(a) or DFO-II can comprise chemical modifications as described herein without limitation, such as, for example, nucleotides having any of Formulae I-VII, stabilization chemistries as described in **Table IV**, or any other combination of modified nucleotides and non-nucleotides as described in the various embodiments herein.

In one embodiment, the palidrome or repeat sequence or modified nucleotide (e.g., nucleotide with a modified base, such as 2-amino purine or a universal base) in Z of DFO constructs having Formula DFO-I, DFO-I(a) and DFO-I(b), comprises chemically modified nucleotides that are able to interact with a portion of the target nucleic acid sequence (e.g., modified base analogs that can form Watson Crick base pairs or non-Watson Crick base pairs).

In one embodiment, a DFO molecule of the invention, for example a DFO having Formula DFO-I or DFO-II, comprises about 15 to about 40 nucleotides (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 nucleotides). In one embodiment, a DFO molecule of the invention comprises one or more chemical modifications. In a non-limiting example, the introduction of chemically modified nucleotides and/or non-nucleotides into nucleic acid molecules of the invention provides a powerful tool in overcoming potential limitations of *in vivo* stability and bioavailability inherent to unmodified RNA molecules that are delivered exogenously. For example, the use of chemically modified nucleic acid molecules can enable a lower dose of a particular nucleic acid molecule for a given therapeutic effect since chemically modified nucleic acid molecules tend to have a longer half-life in serum or in cells or tissues. Furthermore, certain chemical modifications can improve the bioavailability and/or potency of nucleic acid molecules by not only enhancing half-life but also facilitating the targeting of nucleic acid molecules to particular organs, cells or tissues and/or improving cellular uptake of the nucleic acid molecules. Therefore, even if the activity of a chemically modified nucleic acid molecule is reduced in vitro as compared to a native/unmodified nucleic acid molecule, for example when compared to an unmodified RNA molecule, the overall activity of the modified nucleic acid molecule can be greater than the native or unmodified nucleic acid molecule due to improved stability, potency, duration of effect, bioavailability and/or delivery of the molecule.

Multifunctional or Multi-targeted siNA molecules of the Invention

In one embodiment, the invention features siNA molecules comprising multifunctional short interfering nucleic acid (multifunctional siNA) molecules that modulate the expression of one or more genes in a biologic system, such as a cell, tissue, or organism. The multifunctional short interfering nucleic acid (multifunctional siNA) molecules of the invention can target more than one region of the HCV or cellular/host target nucleic acid sequence or can target sequences of more than one distinct target nucleic acid molecules (e.g., HCV RNA or cellular/host RNA targets). The multifunctional siNA molecules of the invention can be chemically synthesized or expressed from transcription units and/or vectors. The multifunctional siNA molecules of the instant invention provide useful reagents and methods for a variety of human applications, therapeutic, diagnostic, agricultural, veterinary, target validation, genomic discovery, genetic engineering and pharmacogenomic applications.

Applicant demonstrates herein that certain oligonucleotides, refered to herein for convenience but not limitation as multifunctional short interfering nucleic acid or multifunctional siNA molecules, are potent mediators of sequence specific regulation of gene expression. The multifunctional siNA molecules of the invention are distinct from other nucleic acid sequences known in the art (e.g., siRNA, miRNA, stRNA, shRNA, antisense oligonucleotides, *etc.*) in that they represent a class of polynucleotide molecules that are designed such that each strand in the multifunctional siNA construct comprises a nucleotide sequence that is complementary to a distinct nucleic acid sequence in one or more target nucleic acid molecules. A single multifunctional siNA molecule (generally a double-stranded molecule) of the invention can thus target more than one (e.g., 2, 3, 4, 5, or more) differing target nucleic acid target molecules. Nucleic acid molecules of the invention can also target more than one (e.g., 2, 3, 4, 5, or more) region of the same target nucleic acid sequence. As such multifunctional siNA molecules of the invention are useful in down regulating or inhibiting the expression of one or more target nucleic acid molecules. For example, a multifunctional siNA molecule of the invention can target nucleic acid molecules encoding a virus or viral proteins and corresponding cellular proteins required for viral infection and/or replication, or differing strains of a particular virus (e.g., HCV). By reducing or inhibiting expression of more than one target nucleic acid molecule with one multifunctional siNA construct, multifunctional siNA molecules of the invention

represent a class of potent therapeutic agents that can provide simultaneous inhibition of multiple targets within a disease or pathogen related pathway. Such simultaneous inhibition can provide synergistic therapeutic treatment strategies without the need for separate preclinical and clinical development efforts or complex regulatory approval process.

Use of multifunctional siNA molecules that target more then one region of a target nucleic acid molecule (e.g., messenger RNA or HCV RNA) is expected to provide potent inhibition of gene expression. For example, a single multifunctional siNA construct of the invention can target both conserved and variable regions of a target nucleic acid molecule (e.g., HCV RNA), thereby allowing down regulation or inhibition of different strain variants or a virus, or splice variants encoded by a single host gene, or allowing for targeting of both coding and non-coding regions of the host target nucleic acid molecule.

Generally, double stranded oligonucleotides are formed by the assembly of two distinct oligonucleotides where the oligonucleotide sequence of one strand is complementary to the oligonucleotide sequence of the second strand; such double stranded oligonucleotides are generally assembled from two separate oligonucleotides (e.g., siRNA). Alternately, a duplex can be formed from a single molecule that folds on itself (e.g., shRNA or short hairpin RNA). These double stranded oligonucleotides are known in the art to mediate RNA interference and all have a common feature wherein only one nucleotide sequence region (guide sequence or the antisense sequence) has complementarity to a target nucleic acid sequence (e.g., HCV or host RNA) and the other strand (sense sequence) comprises nucleotide sequence that is homologous to the target nucleic acid sequence. Generally, the antisense sequence is retained in the active RISC complex and guides the RISC to the target nucleotide sequence by means of complementary base-pairing of the antisense sequence with the target seqeunce for mediating sequence-specific RNA interference. It is known in the art that in some cell culture systems, certain types of unmodified siRNAs can exhibit "off target" effects. It is hypothesized that this off-target effect involves the participation of the sense sequence instead of the antisense sequence of the siRNA in the RISC complex (see for example Schwarz et al., 2003, Cell, 115, 199-208). In this instance the sense sequence is believed to direct the RISC complex to a sequence (off-target sequence) that is distinct from the intended target sequence, resulting in the inhibition of the off-target sequence. In these double stranded nucleic acid molecules, each strand is complementary to a distinct target

nucleic acid sequence. However, the off-targets that are affected by these dsRNAs are not entirely predictable and are non-specific.

Distinct from the double stranded nucleic acid molecules known in the art, the applicants have developed a novel, potentially cost effective and simplified method of down regulating or inhibiting the expression of more than one target nucleic acid sequence using a single multifunctional siNA construct. The multifunctional siNA molecules of the invention are designed to be double-stranded or partially double stranded, such that a portion of each strand or region of the multifunctional siNA is complementary to a target nucleic acid sequence of choice. As such, the multifunctional siNA molecules of the invention are not limited to targeting sequences that are complementary to each other, but rather to any two differing target nucleic acid sequences. Multifunctional siNA molecules of the invention are designed such that each strand or region of the multifunctional siNA molecule, that is complementary to a given target nucleic acid sequence, is of suitable length (*e.g.*, from about 16 to about 28 nucleotides in length, preferably from about 18 to about 28 nucleotides in length) for mediating RNA interference against the target nucleic acid sequence. The complementarity between the target nucleic acid sequence and a strand or region of the multifunctional siNA must be sufficient (at least about 8 base pairs) for cleavage of the target nucleic acid sequence by RNA interference multifunctional siNA of the invention is expected to minimize off-target effects seen with certain siRNA sequences, such as those described in (Schwarz *et al.,* supra).

It has been reported that dsRNAs of length between 29 base pairs and 36 base pairs (Tuschl *et al.,* International PCT Publication No. WO 02/44321) do not mediate RNAi. One reason these dsRNAs are inactive may be the lack of turnover or dissociation of the strand that interacts with the target RNA sequence, such that the RISC complex is not able to efficiently interact with multiple copies of the target RNA resulting in a significant decrease in the potency and efficiency of the RNAi process. Applicant has surprisingly found that the multifunctional siNAs of the invention can overcome this hurdle and are capable of enhancing the efficiency and potency of RNAi process. As such, in certain embodiments of the invention, multifunctional siNAs of length between about 29 to about 36 base pairs can be designed such that, a portion of each strand of the multifunctional siNA molecule comprises a nucleotide sequence region that is complementary to a target nucleic acid of length sufficient to mediate RNAi efficiently

(e.g., about 15 to about 23 base pairs) and a nucleotide sequence region that is not complementary to the target nucleic acid. By having both complementary and non-complementary portions in each strand of the multifunctional siNA, the multifunctional siNA can mediate RNA interference against a target nucleic acid sequence without being prohibitive to turnover or dissociation (*e.g.*, where the length of each strand is too long to mediate RNAi against the respective target nucleic acid sequence). Furthermore, design of multifunctional siNA molecules of the invention with internal overlapping regions allows the multifunctional siNA molecules to be of favorable (decreased) size for mediating RNA interference and of size that is well suited for use as a therapeutic agent (e.g., wherein each strand is independently from about 18 to about 28 nucleotides in length). Non-limiting examples are lillustrated in the enclosed **Figures 16-21 and 42**.

In one embodiment, a multifunctional siNA molecule of the invention comprises a first region and a second region, where the first region of the multifunctional siNA comprises a nucleotide sequence complementary to a nucleic acid sequence of a first target nucleic acid molecule, and the second region of the multifunctional siNA comprises nucleic acid sequence complementary to a nucleic acid sequence of a second target nucleic acid molecule. In one embodiment, a multifunctional siNA molecule of the invention comprises a first region and a second region, where the first region of the multifunctional siNA comprises nucleotide sequence complementary to a nucleic acid sequence of the first region of a target nucleic acid molecule, and the second region of the multifunctional siNA comprises nucleotide sequence complementary to a nucleic acid sequence of a second region of a the target nucleic acid molecule. In another embodiment, the first region and second region of the multifunctional siNA can comprise separate nucleic acid sequences that share some degree of complementarity (e.g., from about 1 to about 10 complementary nucleotides). In certain embodiments, multifunctional siNA constructs comprising separate nucleic acid seqeunces can be readily linked post-synthetically by methods and reagents known in the art and such linked constructs are within the scope of the invention. Alternately, the first region and second region of the multifunctional siNA can comprise a single nucleic acid sequence having some degree of self complementarity, such as in a hairpin or stem-loop structure. Non-limiting examples of such double stranded and hairpin multifunctional short interfering nucleic acids are illustrated in **Figures 16 and 17** respectively. These multifunctional short interfering nucleic acids (multifunctional siNAs) can optionally

include certain overlapping nucleotide sequence where such overlapping nucleotide sequence is present in between the first region and the second region of the multifunctional siNA (see for example **Figures 18 and 19**).

In one embodiment, the invention features a multifunctional short interfering nucleic acid (multifunctional siNA) molecule, wherein each strand of the the multifunctional siNA independently comprises a first region of nucleic acid sequence that is complementary to a distinct target nucleic acid sequence and the second region of nucleotide sequence that is not complementary to the target sequence. The target nucleic acid sequence of each strand is in the same target nucleic acid molecule or different target nucleic acid molecules.

In another embodiment, the multifunctional siNA comprises two strands, where: (a) the first strand comprises a region having sequence complementarity to a target nucleic acid sequence (complementary region 1) and a region having no sequence complementarity to the target nucleotide sequence (non-complementary region 1); (b) the second strand of the multifunction siNA comprises a region having sequence complementarity to a target nucleic acid sequence that is distinct from the target nucleotide sequence complementary to the first strand nucleotide sequence (complementary region 2), and a region having no sequence complementarity to the target nucleotide sequence of complementary region 2 (non-complementary region 2); (c) the complementary region 1 of the first strand comprises a nucleotide sequence that is complementary to a nucleotide sequence in the non-complementary region 2 of the second strand and the complementary region 2 of the second strand comprises a nucleotide sequence that is complementary to a nucleotide sequence in the non-complementary region 1 of the first strand. The target nucleic acid sequence of complementary region 1 and complementary region 2 is in the same target nucleic acid molecule or different target nucleic acid molecules.

In another embodiment, the multifunctional siNA comprises two strands, where: (a) the first strand comprises a region having sequence complementarity to a target nucleic acid sequence derived from a gene (e.g., HCV or host gene) (complementary region 1) and a region having no sequence complementarity to the target nucleotide sequence of complementary region 1 (non-complementary region 1); (b) the second strand of the multifunction siNA comprises a region having sequence complementarity to

a target nucleic acid sequence derived from a gene that is distinct from the gene of complementary region 1 (complementary region 2), and a region having no sequence complementarity to the target nucleotide sequence of complementary region 2 (non-complementary region 2); (c) the complementary region 1 of the first strand comprises a nucleotide sequence that is complementary to a nucleotide sequence in the non-complementary region 2 of the second strand and the complementary region 2 of the second strand comprises a nucleotide sequence that is complementary to a nucleotide sequence in the non-complementary region 1 of the first strand.

In another embodiment, the multifunctional siNA comprises two strands, where: (a) the first strand comprises a region having sequence complementarity to a target nucleic acid sequence derived from a gene (e.g., HCV or host gene) (complementary region 1) and a region having no sequence complementarity to the target nucleotide sequence of complementary region 1 (non-complementary region 1); (b) the second strand of the multifunction siNA comprises a region having sequence complementarity to a target nucleic acid sequence distinct from the target nucleic acid sequence of complementary region 1(complementary region 2), provided, however, that the target nucleic acid sequence for complementary region 1 and target nucleic acid sequence for complementary region 2 are both derived from the same gene, and a region having no sequence complementarity to the target nucleotide sequence of complementary region 2 (non-complementary region 2); (c) the complementary region 1 of the first strand comprises a nucleotide sequence that is complementary to a nucleotide sequence in the non-complementary region 2 of the second strand and the complementary region 2 of the second strand comprises a nucleotide sequence that is complementary to nucleotide sequence in the non-complementary region 1 of the first strand.

In one embodiment, the invention features a multifunctional short interfering nucleic acid (multifunctional siNA) molecule, wherein the multifunctional siNA comprises two complementary nucleic acid sequences in which the first sequence comprises a first region having nucleotide sequence complementary to nucleotide sequence within a target nucleic acid molecule, and in which the second seqeunce comprises a first region having nucleotide sequence complementary to a distinct nucleotide sequence within the same target nucleic acid molecule. Preferably, the first region of the first sequence is also complementary to the nucleotide sequence of the

second region of the second sequence, and where the first region of the second sequence is complementary to the nucleotide sequence of the second region of the first sequence,

In one embodiment, the invention features a multifunctional short interfering nucleic acid (multifunctional siNA) molecule, wherein the multifunctional siNA comprises two complementary nucleic acid sequences in which the first sequence comprises a first region having a nucleotide sequence complementary to a nucleotide sequence within a first target nucleic acid molecule, and in which the second seqeunce comprises a first region having a nucleotide sequence complementary to a distinct nucleotide sequence within a second target nucleic acid molecule. Preferably, the first region of the first sequence is also complementary to the nucleotide sequence of the second region of the second sequence, and where the first region of the second sequence is complementary to the nucleotide sequence of the second region of the first sequence,

In one embodiment, the invention features a multifunctional siNA molecule comprising a first region and a second region, where the first region comprises a nucleic acid sequence having about 18 to about 28 nucleotides complementary to a nucleic acid sequence within a first target nucleic acid molecule, and the second region comprises nucleotide sequence having about 18 to about 28 nucleotides complementary to a distinct nucleic acid sequence within a second target nucleic acid molecule.

In one embodiment, the invention features a multifunctional siNA molecule comprising a first region and a second region, where the first region comprises nucleic acid sequence having about 18 to about 28 nucleotides complementary to a nucleic acid sequence within a target nucleic acid molecule, and the second region comprises nucleotide sequence having about 18 to about 28 nucleotides complementary to a distinct nucleic acid sequence within the same target nucleic acid molecule.

In one embodiment, the invention features a double stranded multifunctional short interfering nucleic acid (multifunctional siNA) molecule, wherein one strand of the multifunctional siNA comprises a first region having nucleotide sequence complementary to a first target nucleic acid sequence, and the second strand comprises a first region having a nucleotide sequence complementary to a second target nucleic acid sequence. The first and second target nucleic acid sequences can be present in separate target nucleic acid molecules or can be different regions within the same target nucleic acid molecule. As such, multifunctional siNA molecules of the invention can be used to

target the expression of different genes, splice variants of the same gene, both mutant and conserved regions of one or more gene transcripts, or both coding and non-coding sequences of the same or differeing genes or gene transcripts.

In one embodiment, a target nucleic acid molecule of the invention encodes a single protein. In another embodiment, a target nucleic acid molecule encodes more than one protein (e.g., 1, 2, 3, 4, 5 or more proteins). As such, a multifunctional siNA construct of the invention can be used to down regulate or inhibit the expression of several proteins. For example, a multifunctional siNA molecule comprising a region in one strand having nucleotide sequence complementarity to a first target nucleic acid sequence derived from a viral genome (e.g., HCV) and the second strand comprising a region with nucleotide sequence complementarity to a second target nucleic acid sequence present in target nucleic acid molecules derived from genes encoding two proteins (e.g., two differing host proteins involved in the HCV life-cycle) can be used to down regulate, inhibit, or shut down a particular biologic pathway by targeting, for example, a viral RNA (e.g., HCV RNA) and one or more host RNAs that are involved in viral infection or the viral life-cycle (e.g., La antigen or interferon regulatory factors).

In another non-limiting example, a multifunctional siNA molecule comprising a region in one strand having a nucleotide sequence complementarity to a first target nucleic acid sequence derived from a target nucleic acid molecule encoding a virus or a viral protein (e.g., HIV) and the second strand comprising a region having a nucleotide sequence complementarity to a second target nucleic acid sequence present in target nucleic acid molecule encoding a cellular protein (e.g., a receptor for the virus, such as CCR5 receptor for HIV) can be used to down regulate, inhibit, or shut down the viral replication and infection by targeting the virus and cellular proteins necessary for viral infection or replication.

In another nonlimiting example, a multifunctional siNA molecule comprising a region in one strand having a nucleotide sequence complementarity to a first target nucleic acid sequence (e.g., conserved sequence) present in a target nucleic acid molecule such as a viral genome (e.g., HCV RNA) and the second strand comprising a region having a nucleotide sequence complementarity to a second target nucleic acid sequence (e.g., conserved sequence) present in target nucleic acid molecule derived from a gene encoding a viral protein (e.g., HCV proteins) to down regulate, inhibit, or shut

down the viral replication and infection by targeting the viral genome and viral encoded proteins necessary for viral infection or replication.

In one embodiment the invention takes advantage of conserved nucleotide sequences present in different strains, isotypes or forms of a virus and genes encoded by these different strains, isotypes and forms of the virus (e.g., HCV). By designing multifunctional siNAs in a manner where one strand includes a sequence that is complementary to target nucleic acid sequence conserved among various strains, isotypes or forms of a virus and the other strand includes sequence that is complementary to target nucleic acid sequence conserved in a protein encoded by the virus, it is possible to selectively and effectively inhibit viral replication or infection using a single multifunctional siNA.

In one embodiment, a multifunctional short interfering nucleic acid (multifunctional siNA) of the invention comprises a first region and a second region, wherein the first region comprises nucleotide sequence complementary to a HCV viral RNA of a first viral strain and the second region comprises nucleotide sequence complementary to a HCV viral RNA of a second viral strain. In one embodiment, the first and second regions can comprise nucleotide sequence complementary to shared or conserved RNA sequences of differing viral strains or classes or viral strains.

In one embodiment, a multifunctional short interfering nucleic acid (multifunctional siNA) of the invention comprises a first region and a second region, wherein the first region comprises a nucleotide sequence complementary to a HCV viral RNA encoding one or more HCV viruses (e.g., one or more strains of HCV) and the second region comprises a nucleotide sequence complementary to a viral RNA encoding one or more interferon agonist proteins. In one embodiment, the first region can comprise a nucleotide sequence complementary to shared or conserved RNA sequences of differing HCV viral strains or classes of HCV viral strains. Non-limiting example of interferon agonist proteins include any protein that is capable of inhibition or suppressing RNA silencing (e.g., RNA binding proteins such as E3L or NS1 or equivalents thereof, see for example Li *et al.*, 2004, *PNAS*, 101, 1350-1355)

In one embodiment, a multifunctional short interfering nucleic acid (multifunctional siNA) of the invention comprises a first region and a second region, wherein the first region comprises nucleotide sequence complementary to a HCV viral

RNA and the second region comprises nucleotide sequence complementary to a cellular RNA that is involved in HCV viral infection and/or replication. Non-limiting examples of cellular RNAs involved in viral infection and/or replication include cellular receptors, cell surface molecules, cellular enzymes, cellular transcription factors, and/or cytokines, second messengers, and cellular accessory molecules including, but not limited to, La antigen, FAS, interferon agonsit proteins (e.g., E3L or NS1 or equivalents thereof, see for example Li *et al.,* 2004, *PNAS,* 101, 1350-1355), interferon regulatory factors (IRFs); cellular PKR protein kinase (PKR); human eukaryotic initiation factors 2B (elF2B gamma and/or elF2gamma); human DEAD Box protein (DDX3); and cellular proteins that bind to the poly(U) tract of the HCV 3'-UTR, such as polypyrimidine tract-binding protein.

In one embodiment, a double stranded multifunctional siNA molecule of the invention comprises a structure having Formula MF-I:

$$\begin{array}{l} \mathbf{5'\text{-}p\text{-}X\ Z\ X'\text{-}3'} \\ \quad\ \mathbf{3'\text{-}Y'\ Z\ Y\text{-}p\text{-}5'} \end{array}$$

wherein each 5'-p-XZX'-3' and 5'-p-YZY'-3' are independently an oligonucleotide of length about 20 nucleotides to about 300 nucleotides, preferably about 20 to about 200 nucleotides, about 20 to about 100 nucleotides, about 20 to about 40 nucleotides, about 20 to about 40 nucleotides, about 24 to about 38 nucleotides, or about 26 to about 38 nucleotides; XZ comprises a nucleic acid sequence that is complementary to a first HCV target nucleic acid sequence; YZ is an oligonucleotide comprising nucleic acid sequence that is complementary to a second HCV target nucleic acid sequence; Z comprises nucleotide sequence of length about 1 to about 24 nucleotides (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 nucleotides) that is self complementary; X comprises nucleotide sequence of length about 1 to about 100 nucleotides, preferably about 1 to about 21 nucleotides (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 nucleotides) that is complementary to nucleotide sequence present in region Y'; Y comprises nucleotide sequence of length about 1 to about 100 nucleotides, preferably about 1 to about 21 nucleotides (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 nucleotides) that is complementary to nucleotide sequence present in region X'; each p comprises a terminal phosphate group that is independently present or absent; each XZ and YZ is

independently of length sufficient to stably interact (i.e., base pair) with the first and second target nucleic acid sequence, respectively, or a portion thereof. For example, each sequence X and Y can independently comprise sequence from about 12 to about 21 or more nucleotides in length (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more) that is complementary to a target nucleotide sequence in different target nucleic acid molecules, such as target RNAs or a portion thereof. In another non-limiting example, the length of the nucleotide sequence of X and Z together that is complementary to the first HCV target nucleic acid sequence or a portion thereof is from about 12 to about 21 or more nucleotides (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more). In another non-limiting example, the length of the nucleotide sequence of Y and Z together, that is complementary to the second HCV target nucleic acid sequence or a portion thereof is from about 12 to about 21 or more nucleotides (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more). In one embodiment, the first HCV target nucleic acid sequence and the second HCV target nucleic acid sequence are present in the same target nucleic acid molecule (e.g., HCV RNA or host RNA). In another embodiment, the first HCV target nucleic acid sequence and the second HCV target nucleic acid sequence are present in different target nucleic acid molecules (e.g., HCV RNA and host RNA). In one embodiment, Z comprises a palindrome or a repeat sequence. In one embodiment, the lengths of oligonucleotides X and X' are identical. In another embodiment, the lengths of oligonucleotides X and X' are not identical. In one embodiment, the lengths of oligonucleotides Y and Y' are identical. In another embodiment, the lengths of oligonucleotides Y and Y' are not identical. In one embodiment, the double stranded oligonucleotide construct of Formula I(a) includes one or more, specifically 1, 2, 3 or 4, mismatches, to the extent such mismatches do not significantly diminish the ability of the double stranded oligonucleotide to inhibit target gene expression.

In one embodiment, a multifunctional siNA molecule of the invention comprises a structure having Formula MF-II:

5'-p-X X'-3'
3'-Y' Y-p-5'

wherein each 5'-p-XX'-3' and 5'-p-YY'-3' are independently an oligonucleotide of length about 20 nucleotides to about 300 nucleotides, preferably about 20 to about 200 nucleotides, about 20 to about 100 nucleotides, about 20 to about 40 nucleotides, about

20 to about 40 nucleotides, about 24 to about 38 nucleotides, or about 26 to about 38 nucleotides; X comprises a nucleic acid sequence that is complementary to a first target nucleic acid sequence; Y is an oligonucleotide comprising nucleic acid sequence that is complementary to a second target nucleic acid sequence; X comprises a nucleotide sequence of length about 1 to about 100 nucleotides, preferably about 1 to about 21 nucleotides (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 nucleotides) that is complementary to nucleotide sequence present in region Y'; Y comprises nucleotide sequence of length about 1 to about 100 nucleotides, preferably about 1 to about 21 nucleotides (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 nucleotides) that is complementary to nucleotide sequence present in region X'; each p comprises a terminal phosphate group that is independently present or absent; each X and Y independently is of length sufficient to stably interact (i.e., base pair) with the first and second target nucleic acid sequence, respectively, or a portion thereof. For example, each sequence X and Y can independently comprise sequence from about 12 to about 21 or more nucleotides in length (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more) that is complementary to a target nucleotide sequence in different target nucleic acid molecules, such as HCV target RNAs or a portion thereof. In one embodiment, the first HCV target nucleic acid sequence and the second HCV target nucleic acid sequence are present in the same target nucleic acid molecule (e.g., HCV RNA or host RNA). In another embodiment, the first HCV target nucleic acid sequence and the second HCV target nucleic acid sequence are present in different target nucleic acid molecules (e.g., HCV RNA and host RNA). In one embodiment, Z comprises a palindrome or a repeat sequence. In one embodiment, the lengths of oligonucleotides X and X' are identical. In another embodiment, the lengths of oligonucleotides X and X' are not identical. In one embodiment, the lengths of oligonucleotides Y and Y' are identical. In another embodiment, the lengths of oligonucleotides Y and Y' are not identical. In one embodiment, the double stranded oligonucleotide construct of Formula I(a) includes one or more, specifically 1, 2, 3 or 4, mismatches, to the extent such mismatches do not significantly diminish the ability of the double stranded oligonucleotide to inhibit target gene expression.

In one embodiment, a multifunctional siNA molecule of the invention comprises a structure having Formula MF-III:

$$\begin{array}{cc} \mathbf{X} & \mathbf{X'} \\ \multicolumn{2}{c}{\mathbf{Y'\text{-}W\text{-}Y}} \end{array}$$

wherein each X, X', Y, and Y' is independently an oligonucleotide of length about 15 nucleotides to about 50 nucleotides, preferably about 18 to about 40 nucleotides, or about 19 to about 23 nucleotides; X comprises nucleotide sequence that is complementary to nucleotide sequence present in region Y'; X' comprises nucleotide sequence that is complementary to nucleotide sequence present in region Y; each X and X' is independently of length sufficient to stably interact (i.e., base pair) with a first and a second HCV target nucleic acid sequence, respectively, or a portion thereof; W represents a nucleotide or non-nucleotide linker that connects sequences Y' and Y; and the multifunctional siNA directs cleavage of the first and second HCV target sequence via RNA interference. In one embodiment, the first HCV target nucleic acid sequence and the second HCV target nucleic acid sequence are present in the same target nucleic acid molecule (e.g., HCV RNA or host RNA). In another embodiment, the first HCV target nucleic acid sequence and the second HCV target nucleic acid sequence are present in different target nucleic acid molecules (e.g., HCV RNA and host RNA). In one embodiment, region W connects the 3'-end of sequence Y' with the 3'-end of sequence Y. In one embodiment, region W connects the 3'-end of sequence Y' with the 5'-end of sequence Y. In one embodiment, region W connects the 5'-end of sequence Y' with the 5'-end of sequence Y. In one embodiment, region W connects the 5'-end of sequence Y' with the 3'-end of sequence Y. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence X. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence X'. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence Y. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence Y'. In one embodiment, W connects sequences Y and Y' via a biodegradable linker. In one embodiment, W further comprises a conjugate, lable, aptamer, ligand, lipid, or polymer.

In one embodiment, a multifunctional siNA molecule of the invention comprises a structure having Formula MF-IV:

$$\begin{array}{cc} \mathbf{X} & \mathbf{X'} \\ \multicolumn{2}{c}{\mathbf{Y'\text{-}W\text{-}Y}} \end{array}$$

wherein each X, X', Y, and Y' is independently an oligonucleotide of length about 15 nucleotides to about 50 nucleotides, preferably about 18 to about 40 nucleotides, or about 19 to about 23 nucleotides; X comprises nucleotide sequence that is complementary to nucleotide sequence present in region Y'; X' comprises nucleotide sequence that is complementary to nucleotide sequence present in region Y; each Y and Y' is independently of length sufficient to stably interact (i.e., base pair) with a first and a second HCV target nucleic acid sequence, respectively, or a portion thereof; W represents a nucleotide or non-nucleotide linker that connects sequences Y' and Y; and the multifunctional siNA directs cleavage of the first and second HCV target sequence via RNA interference. In one embodiment, the first HCV target nucleic acid sequence and the second HCV target nucleic acid sequence are present in the same target nucleic acid molecule (e.g., HCV RNA or host RNA). In another embodiment, the first HCV target nucleic acid sequence and the second HCV target nucleic acid sequence are present in different target nucleic acid molecules (e.g., HCV RNA and host RNA). In one embodiment, region W connects the 3'-end of sequence Y' with the 3'-end of sequence Y. In one embodiment, region W connects the 3'-end of sequence Y' with the 5'-end of sequence Y. In one embodiment, region W connects the 5'-end of sequence Y' with the 5'-end of sequence Y. In one embodiment, region W connects the 5'-end of sequence Y' with the 3'-end of sequence Y. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence X. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence X'. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence Y. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence Y'. In one embodiment, W connects sequences Y and Y' via a biodegradable linker. In one embodiment, W further comprises a conjugate, lable, aptamer, ligand, lipid, or polymer.

In one embodiment, a multifunctional siNA molecule of the invention comprises a structure having Formula MF-V:

$$\begin{array}{cc} \mathbf{X} & \mathbf{X'} \\ \multicolumn{2}{c}{\mathbf{Y'\text{-}W\text{-}Y}} \end{array}$$

wherein each X, X', Y, and Y' is independently an oligonucleotide of length about 15 nucleotides to about 50 nucleotides, preferably about 18 to about 40 nucleotides, or about 19 to about 23 nucleotides; X comprises nucleotide sequence that is complementary to

nucleotide sequence present in region Y'; X' comprises nucleotide sequence that is complementary to nucleotide sequence present in region Y; each X, X', Y, or Y' is independently of length sufficient to stably interact (i.e., base pair) with a first, second, third, or fourth HCV target nucleic acid sequence, respectively, or a portion thereof; W represents a nucleotide or non-nucleotide linker that connects sequences Y' and Y; and the multifunctional siNA directs cleavage of the first, second, third, and/or fourth target sequence via RNA interference. In one embodiment, the first, second, third and fourth HCV target nucleic acid sequence are all present in the same target nucleic acid molecule (e.g., HCV RNA or host RNA). In another embodiment, the first, second, third and fourth HCV target nucleic acid sequence are independently present in different target nucleic acid molecules (e.g., HCV RNA and host RNA). In one embodiment, region W connects the 3'-end of sequence Y' with the 3'-end of sequence Y. In one embodiment, region W connects the 3'-end of sequence Y' with the 5'-end of sequence Y. In one embodiment, region W connects the 5'-end of sequence Y' with the 5'-end of sequence Y. In one embodiment, region W connects the 5'-end of sequence Y' with the 3'-end of sequence Y. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence X. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence X'. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence Y. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence Y'. In one embodiment, W connects sequences Y and Y' via a biodegradable linker. In one embodiment, W further comprises a conjugate, lable, aptamer, ligand, lipid, or polymer.

In one embodiment, regions X and Y of multifunctional siNA molecule of the invention (e.g., having any of Formula MF-I - MF-V), are complementary to different target nucleic acid sequences that are portions of the same target nucleic acid molecule. In one embodiment, such target nucleic acid sequences are at different locations within the coding region of a RNA transcript. In one embodiment, such target nucleic acid sequences comprise coding and non-coding regions of the same RNA transcript. In one embodiment, such target nucleic acid sequences comprise regions of alternately spliced transcripts or precursors of such alternately spliced transcripts.

In one embodiment, a multifunctional siNA molecule having any of Formula MF-I - MF-V can comprise chemical modifications as described herein without limitation, such as, for example, nucleotides having any of Formulae I-VII described herein,

stabilization chemistries as described in **Table IV**, or any other combination of modified nucleotides and non-nucleotides as described in the various embodiments herein.

In one embodiment, the palidrome or repeat sequence or modified nucleotide (e.g., nucleotide with a modified base, such as 2-amino purine or a universal base) in Z of multifunctional siNA constructs having Formula MF-I or MF-II comprises chemically modified nucleotides that are able to interact with a portion of the target nucleic acid sequence (e.g., modified base analogs that can form Watson Crick base pairs or non-Watson Crick base pairs).

In one embodiment, a multifunctional siNA molecule of the invention, for example each strand of a multifunctional siNA having MF-I – MF-V, independently comprises about 15 to about 40 nucleotides (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 nucleotides). In one embodiment, a multifunctional siNA molecule of the invention comprises one or more chemical modifications. In a non-limiting example, the introduction of chemically modified nucleotides and/or non-nucleotides into nucleic acid molecules of the invention provides a powerful tool in overcoming potential limitations of *in vivo* stability and bioavailability inherent to unmodified RNA molecules that are delivered exogenously. For example, the use of chemically modified nucleic acid molecules can enable a lower dose of a particular nucleic acid molecule for a given therapeutic effect since chemically modified nucleic acid molecules tend to have a longer half-life in serum or in cells or tissues. Furthermore, certain chemical modifications can improve the bioavailability and/or potency of nucleic acid molecules by not only enhancing half-life but also facilitating the targeting of nucleic acid molecules to particular organs, cells or tissues and/or improving cellular uptake of the nucleic acid molecules. Therefore, even if the activity of a chemically modified nucleic acid molecule is reduced *in vitro* as compared to a native/unmodified nucleic acid molecule, for example when compared to an unmodified RNA molecule, the overall activity of the modified nucleic acid molecule can be greater than the native or unmodified nucleic acid molecule due to improved stability, potency, duration of effect, bioavailability and/or delivery of the molecule.

In another embodiment, the invention features multifunctional siNAs, wherein the multifunctional siNAs are assembled from two separate double-stranded siNAs, with one of the ends of each sense strand is tethered to the end of the sense strand of the other

siNA molecule, such that the two antisense siNA strands are annealed to their corresponding sense strand that are tethered to each other at one end (see **Figure 42**). The tethers or linkers can be nucleotide-based linkers or non-nucleotide based linkers as generally known in the art and as described herein.

In one embodiment, the invention features a multifunctional siNA, wherein the multifunctional siNA is assembled from two separate double-stranded siNAs, with the 5'-end of one sense strand of the siNA is tethered to the 5'- end of the sense strand of the other siNA molecule, such that the 5'-ends of the two antisense siNA strands, annealed to their corresponding sense strand that are tethered to each other at one end, point away (in the opposite direction) from each other (see **Figure 42 (A)**). The tethers or linkers can be nucleotide-based linkers or non-nucleotide based linkers as generally known in the art and as described herein.

In one embodiment, the invention features a multifunctional siNA, wherein the multifunctional siNA is assembled from two separate double-stranded siNAs, with the 3'-end of one sense strand of the siNA is tethered to the 3'- end of the sense strand of the other siNA molecule, such that the 5'-ends of the two antisense siNA strands, annealed to their corresponding sense strand that are tethered to each other at one end, face each other (see **Figure 42 (B)**). The tethers or linkers can be nucleotide-based linkers or non-nucleotide based linkers as generally known in the art and as described herein.

In one embodiment, the invention features a multifunctional siNA, wherein the multifunctional siNA is assembled from two separate double-stranded siNAs, with the 5'-end of one sense strand of the siNA is tethered to the 3'- end of the sense strand of the other siNA molecule, such that the 5'-end of the one of the antisense siNA strands annealed to their corresponding sense strand that are tethered to each other at one end, faces the 3'-end of the other antisense strand (see **Figure 42 (C-D)**). The tethers or linkers can be nucleotide-based linkers or non-nucleotide based linkers as generally known in the art and as described herein.

In one embodiment, the invention features a multifunctional siNA, wherein the multifunctional siNA is assembled from two separate double-stranded siNAs, with the 5'-end of one antisense strand of the siNA is tethered to the 3'- end of the antisense strand of the other siNA molecule, such that the 5'-end of the one of the sense siNA strands annealed to their corresponding antisense sense strand that are tethered to each

other at one end, faces the 3'-end of the other sense strand (see **Figure 42 (G-H)**). In one embodiment, the linkage between the 5'-end of the first antisense strand and the 3'-end of the second antisense strand is designed in such a way as to be readily cleavable (e.g., biodegradable linker) such that the 5'end of each antisense strand of the multifunctional siNA has a free 5'-end suitable to mediate RNA interefence-based cleavage of the target RNA. The tethers or linkers can be nucleotide-based linkers or non-nucleotide based linkers as generally known in the art and as described herein.

In one embodiment, the invention features a multifunctional siNA, wherein the multifunctional siNA is assembled from two separate double-stranded siNAs, with the 5'-end of one antisense strand of the siNA is tethered to the 5'- end of the antisense strand of the other siNA molecule, such that the 3'-end of the one of the sense siNA strands annealed to their corresponding antisense sense strand that are tethered to each other at one end, faces the 3'-end of the other sense strand (see **Figure 42 (E)**). In one embodiment, the linkage between the 5'-end of the first antisense strand and the 5'-end of the second antisense strand is designed in such a way as to be readily cleavable (e.g., biodegradable linker) such that the 5'end of each antisense strand of the multifunctional siNA has a free 5'-end suitable to mediate RNA interefence-based cleavage of the target RNA. The tethers or linkers can be nucleotide-based linkers or non-nucleotide based linkers as generally known in the art and as described herein.

In one embodiment, the invention features a multifunctional siNA, wherein the multifunctional siNA is assembled from two separate double-stranded siNAs, with the 3'-end of one antisense strand of the siNA is tethered to the 3'- end of the antisense strand of the other siNA molecule, such that the 5'-end of the one of the sense siNA strands annealed to their corresponding antisense sense strand that are tethered to each other at one end, faces the 3'-end of the other sense strand (see **Figure 42 (F)**). In one embodiment, the linkage between the 5'-end of the first antisense strand and the 5'-end of the second antisense strand is designed in such a way as to be readily cleavable (e.g., biodegradable linker) such that the 5'end of each antisense strand of the multifunctional siNA has a free 5'-end suitable to mediate RNA interefence-based cleavage of the target RNA. The tethers or linkers can be nucleotide-based linkers or non-nucleotide based linkers as generally known in the art and as described herein.

In any of the above embodiments, a first target nucleic acid sequence or second target nucleic acid sequence can independently comprise HCV RNA or a portion thereof or a polynucleotide coding or non-coding sequence of cellular or host target that is invoved in HCV infection or replication, or disease processes associated with HCV infection such as such as cellular receptors, cell surface molecules, cellular enzymes, cellular transcription factors, and/or cytokines, second messengers, and cellular accessory molecules including, but not limited to, La antigen (see for example Costa-Mattioli *et al.,* 2004, *Mol Cell Biol.,* 24, 6861-70, e.g., Genbank Accession No. NM_003142); FAS (e.g., Genbank Accession No. NM_000043) or FAS ligand (e.g., Genbank Accession No. NM_000639); interferon regulatory factors (IRFs; e.g., Genbank Accession No. AF082503.1); cellular PKR protein kinase (e.g., Genbank Accession No. XM_002661.7); human eukaryotic initiation factors 2B (elF2Bgamma; e.g., Genbank Accession No. AF256223, and/or elF2gamma; e.g., Genbank Accession No. NM_006874.1); human DEAD Box protein (DDX3; e.g., Genbank Accession No. XM_018021.2); and cellular proteins that bind to the poly(U) tract of the HCV 3'-UTR, such as polypyrimidine tract-binding protein (e.g., Genbank Accession Nos. NM_031991.1 and XM_042972.3). In one embodiment, the first HCV target nucleic acid sequence is a HCV RNA or a portion thereof and the second HCV target nucleic acid sequence is a HCV RNA of a portion thereof. In one embodiment, the first HCV target nucleic acid sequence is a HCV RNA or a portion thereof and the second HCV target nucleic acid sequence is a host RNA or a portion thereof. In one embodiment, the first HCV target nucleic acid sequence is a host RNA or a portion thereof and the second HCV target nucleic acid sequence is a host RNA or a portion thereof. In one embodiment, the first HCV target nucleic acid sequence is a host RNA or a portion thereof and the second HCV target nucleic acid sequence is a HCV RNA or a portion thereof.

Synthesis of Nucleic Acid Molecules

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small nucleic acid motifs ("small" refers to nucleic acid motifs no more than 100 nucleotides in length, preferably no more than 80 nucleotides in length, and most preferably no more than 50 nucleotides in length; *e.g.*, individual siNA oligonucleotide sequences or siNA sequences synthesized in tandem) are preferably used for exogenous

delivery. The simple structure of these molecules increases the ability of the nucleic acid to invade targeted regions of protein and/or RNA structure. Exemplary molecules of the instant invention are chemically synthesized, and others can similarly be synthesized.

Oligonucleotides (*e.g.*, certain modified oligonucleotides or portions of oligonucleotides lacking ribonucleotides) are synthesized using protocols known in the art, for example as described in Caruthers *et al.*, 1992, *Methods in Enzymology* 211, 3-19, Thompson *et al.*, International PCT Publication No. WO 99/54459, Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684, Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, Brennan *et al.*, 1998, *Biotechnol Bioeng.*, 61, 33-45, and Brennan, U.S. Pat. No. 6,001,311. All of these references are incorporated herein by reference. The synthesis of oligonucleotides makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μmol scale protocol with a 2.5 min coupling step for 2'-O-methylated nucleotides and a 45 second coupling step for 2'-deoxy nucleotides or 2'-deoxy-2'-fluoro nucleotides. **Table V** outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μmol scale can be performed on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 μL of 0.11 M = 6.6 μmol) of 2'-O-methyl phosphoramidite and a 105-fold excess of S-ethyl tetrazole (60 μL of 0.25 M = 15 μmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 22-fold excess (40 μL of 0.11 M = 4.4 μmol) of deoxy phosphoramidite and a 70-fold excess of S-ethyl tetrazole (40 μL of 0.25 M = 10 μmol) can be used in each coupling cycle of deoxy residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); and oxidation solution is 16.9 mM I_2, 49 mM pyridine, 9% water in THF (PerSeptive Biosystems, Inc.). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained

from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide, 0.05 M in acetonitrile) is used.

Deprotection of the DNA-based oligonucleotides is performed as follows: the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aqueous methylamine (1 mL) at 65 °C for 10 minutes. After cooling to –20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder.

The method of synthesis used for RNA including certain siNA molecules of the invention follows the procedure as described in Usman *et al.*, 1987, *J. Am. Chem. Soc.*, 109, 7845; Scaringe *et al.,* 1990, *Nucleic Acids Res.*, 18, 5433; and Wincott *et al.,* 1995, *Nucleic Acids Res.* 23, 2677-2684 Wincott *et al.,* 1997, *Methods Mol. Bio.*, 74, 59, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μmol scale protocol with a 7.5 min coupling step for alkylsilyl protected nucleotides and a 2.5 min coupling step for 2'-O-methylated nucleotides. **Table V** outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μmol scale can be done on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 μL of 0.11 M = 6.6 μmol) of 2'-O-methyl phosphoramidite and a 75-fold excess of S-ethyl tetrazole (60 μL of 0.25 M = 15 μmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 66-fold excess (120 μL of 0.11 M = 13.2 μmol) of alkylsilyl (ribo) protected phosphoramidite and a 150-fold excess of S-ethyl tetrazole (120 μL of 0.25 M = 30 μmol) can be used in each coupling cycle of ribo residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and

10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution is 16.9 mM I_2, 49 mM pyridine, 9% water in THF (PerSeptive Biosystems, Inc.). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide0.05 M in acetonitrile) is used.

Deprotection of the RNA is performed using either a two-pot or one-pot protocol. For the two-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to –20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder. The base deprotected oligoribonucleotide is resuspended in anhydrous TEA/HF/NMP solution (300 μL of a solution of 1.5 mL N-methylpyrrolidinone, 750 μL TEA and 1 mL TEA•3HF to provide a 1.4 M HF concentration) and heated to 65 °C. After 1.5 h, the oligomer is quenched with 1.5 M NH_4HCO_3.

Alternatively, for the one-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 33% ethanolic methylamine/DMSO: 1/1 (0.8 mL) at 65 °C for 15 minutes. The vial is brought to room temperature TEA•3HF (0.1 mL) is added and the vial is heated at 65 °C for 15 minutes. The sample is cooled at –20 °C and then quenched with 1.5 M NH_4HCO_3.

For purification of the trityl-on oligomers, the quenched NH_4HCO_3 solution is loaded onto a C-18 containing cartridge that had been prewashed with acetonitrile followed by 50 mM TEAA. After washing the loaded cartridge with water, the RNA is detritylated with 0.5% TFA for 13 minutes. The cartridge is then washed again with water, salt exchanged with 1 M NaCl and washed with water again. The oligonucleotide is then eluted with 30% acetonitrile.

The average stepwise coupling yields are typically >98% (Wincott *et al.,* 1995 *Nucleic Acids Res.* 23, 2677-2684). Those of ordinary skill in the art will recognize that the scale of synthesis can be adapted to be larger or smaller than the example described above including but not limited to 96-well format.

Alternatively, the nucleic acid molecules of the present invention can be synthesized separately and joined together post-synthetically, for example, by ligation (Moore *et al.,* 1992, *Science* 256, 9923; Draper *et al.,* International PCT publication No. WO 93/23569; Shabarova *et al.,* 1991, *Nucleic Acids Research* 19, 4247; Bellon *et al.,* 1997, *Nucleosides & Nucleotides*, 16, 951; Bellon *et al.,* 1997, *Bioconjugate Chem.* 8, 204), or by hybridization following synthesis and/or deprotection.

The siNA molecules of the invention can also be synthesized via a tandem synthesis methodology as described in Example 1 herein, wherein both siNA strands are synthesized as a single contiguous oligonucleotide fragment or strand separated by a cleavable linker which is subsequently cleaved to provide separate siNA fragments or strands that hybridize and permit purification of the siNA duplex. The linker can be a polynucleotide linker or a non-nucleotide linker. The tandem synthesis of siNA as described herein can be readily adapted to both multiwell/multiplate synthesis platforms such as 96 well or similarly larger multi-well platforms. The tandem synthesis of siNA as described herein can also be readily adapted to large scale synthesis platforms employing batch reactors, synthesis columns and the like.

A siNA molecule can also be assembled from two distinct nucleic acid strands or fragments wherein one fragment includes the sense region and the second fragment includes the antisense region of the RNA molecule.

The nucleic acid molecules of the present invention can be modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-*C*-allyl, 2'-fluoro, 2'-*O*-methyl, 2'-H (for a review see Usman and Cedergren, 1992, *TIBS* 17, 34; Usman *et al.,* 1994, *Nucleic Acids Symp. Ser.* 31, 163). siNA constructs can be purified by gel electrophoresis using general methods or can be purified by high pressure liquid chromatography (HPLC; see Wincott *et al., supra*, the totality of which is hereby incorporated herein by reference) and re-suspended in water.

In another aspect of the invention, siNA molecules of the invention are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules.

Optimizing Activity of the nucleic acid molecule of the invention.

Chemically synthesizing nucleic acid molecules with modifications (base, sugar and/or phosphate) can prevent their degradation by serum ribonucleases, which can increase their potency (see *e.g.*, Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 *Nature* 344, 565; Pieken *et al.*, 1991, *Science* 253, 314; Usman and Cedergren, 1992, *Trends in Biochem. Sci.* 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162; Sproat, U.S. Pat. No. 5,334,711; Gold *et al.*, U.S. Pat. No. 6,300,074; and Burgin *et al.*, *supra;* all of which are incorporated by reference herein). All of the above references describe various chemical modifications that can be made to the base, phosphate and/or sugar moieties of the nucleic acid molecules described herein. Modifications that enhance their efficacy in cells, and removal of bases from nucleic acid molecules to shorten oligonucleotide synthesis times and reduce chemical requirements are desired.

There are several examples in the art describing sugar, base and phosphate modifications that can be introduced into nucleic acid molecules with significant enhancement in their nuclease stability and efficacy. For example, oligonucleotides are modified to enhance stability and/or enhance biological activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-*C*-allyl, 2'-fluoro, 2'-*O*-methyl, 2'-O-allyl, 2'-H, nucleotide base modifications (for a review see Usman and Cedergren, 1992, *TIBS.* 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163; Burgin *et al.*, 1996, *Biochemistry*, 35, 14090). Sugar modification of nucleic acid molecules have been extensively described in the art (see Eckstein *et al.*, *International Publication* PCT No. WO 92/07065; Perrault *et al. Nature*, 1990, *344*, 565-568; Pieken *et al. Science*, 1991, *253*, 314-317; Usman and Cedergren, *Trends in Biochem. Sci.*, 1992, *17*, 334-339;

Usman *et al.* *International Publication* PCT No. WO 93/15187; Sproat, *U.S. Pat.* No. 5,334,711 and Beigelman *et al.*, 1995, *J. Biol. Chem.*, 270, 25702; Beigelman *et al.*, International PCT publication No. WO 97/26270; Beigelman *et al.*, U.S. Pat. No. 5,716,824; Usman *et al.*, U.S. Pat. No. 5,627,053; Woolf *et al.*, International PCT Publication No. WO 98/13526; Thompson *et al.*, USSN 60/082,404 which was filed on April 20, 1998; Karpeisky *et al.*, 1998, *Tetrahedron Lett.*, 39, 1131; Earnshaw and Gait, 1998, *Biopolymers (Nucleic Acid Sciences)*, 48, 39-55; Verma and Eckstein, 1998, *Annu. Rev. Biochem.*, 67, 99-134; and Burlina *et al.*, 1997, *Bioorg. Med. Chem.*, 5, 1999-2010; all of the references are hereby incorporated in their totality by reference herein). Such publications describe general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into nucleic acid molecules without modulating catalysis, and are incorporated by reference herein. In view of such teachings, similar modifications can be used as described herein to modify the siNA nucleic acid molecules of the instant invention so long as the ability of siNA to promote RNAi is cells is not significantly inhibited.

While chemical modification of oligonucleotide internucleotide linkages with phosphorothioate, phosphorodithioate, and/or 5'-methylphosphonate linkages improves stability, excessive modifications can cause some toxicity or decreased activity. Therefore, when designing nucleic acid molecules, the amount of these internucleotide linkages should be minimized. The reduction in the concentration of these linkages should lower toxicity, resulting in increased efficacy and higher specificity of these molecules.

Short interfering nucleic acid (siNA) molecules having chemical modifications that maintain or enhance activity are provided. Such a nucleic acid is also generally more resistant to nucleases than an unmodified nucleic acid. Accordingly, the *in vitro* and/or *in vivo* activity should not be significantly lowered. In cases in which modulation is the goal, therapeutic nucleic acid molecules delivered exogenously should optimally be stable within cells until translation of the target RNA has been modulated long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Improvements in the chemical synthesis of RNA and DNA (Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677; Caruthers *et al.*, 1992, *Methods in Enzymology* 211, 3-19 (incorporated by reference herein)) have expanded the

ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability, as described above.

In one embodiment, nucleic acid molecules of the invention include one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) G-clamp nucleotides. A G-clamp nucleotide is a modified cytosine analog wherein the modifications confer the ability to hydrogen bond both Watson-Crick and Hoogsteen faces of a complementary guanine within a duplex, see for example Lin and Matteucci, 1998, *J. Am. Chem. Soc.,* 120, 8531-8532. A single G-clamp analog substitution within an oligonucleotide can result in substantially enhanced helical thermal stability and mismatch discrimination when hybridized to complementary oligonucleotides. The inclusion of such nucleotides in nucleic acid molecules of the invention results in both enhanced affinity and specificity to nucleic acid targets, complementary sequences, or template strands. In another embodiment, nucleic acid molecules of the invention include one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) LNA "locked nucleic acid" nucleotides such as a 2', 4'-C methylene bicyclo nucleotide (see for example Wengel *et al.,* International PCT Publication No. WO 00/66604 and WO 99/14226).

In another embodiment, the invention features conjugates and/or complexes of siNA molecules of the invention. Such conjugates and/or complexes can be used to facilitate delivery of siNA molecules into a biological system, such as a cell. The conjugates and complexes provided by the instant invention can impart therapeutic activity by transferring therapeutic compounds across cellular membranes, altering the pharmacokinetics, and/or modulating the localization of nucleic acid molecules of the invention. The present invention encompasses the design and synthesis of novel conjugates and complexes for the delivery of molecules, including, but not limited to, small molecules, lipids, cholesterol, phospholipids, nucleosides, nucleotides, nucleic acids, antibodies, toxins, negatively charged polymers and other polymers, for example proteins, peptides, hormones, carbohydrates, polyethylene glycols, or polyamines, across cellular membranes. In general, the transporters described are designed to be used either individually or as part of a multi-component system, with or without degradable linkers. These compounds are expected to improve delivery and/or localization of nucleic acid molecules of the invention into a number of cell types originating from different tissues, in the presence or absence of serum (see Sullenger and Cech, U.S. Pat. No. 5,854,038). Conjugates of the molecules described herein can be attached to biologically active

molecules via linkers that are biodegradable, such as biodegradable nucleic acid linker molecules.

The term "biodegradable linker" as used herein, refers to a nucleic acid or non-nucleic acid linker molecule that is designed as a biodegradable linker to connect one molecule to another molecule, for example, a biologically active molecule to a siNA molecule of the invention or the sense and antisense strands of a siNA molecule of the invention. The biodegradable linker is designed such that its stability can be modulated for a particular purpose, such as delivery to a particular tissue or cell type. The stability of a nucleic acid-based biodegradable linker molecule can be modulated by using various chemistries, for example combinations of ribonucleotides, deoxyribonucleotides, and chemically-modified nucleotides, such as 2'-O-methyl, 2'-fluoro, 2'-amino, 2'-O-amino, 2'-C-allyl, 2'-O-allyl, and other 2'-modified or base modified nucleotides. The biodegradable nucleic acid linker molecule can be a dimer, trimer, tetramer or longer nucleic acid molecule, for example, an oligonucleotide of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length, or can comprise a single nucleotide with a phosphorus-based linkage, for example, a phosphoramidate or phosphodiester linkage. The biodegradable nucleic acid linker molecule can also comprise nucleic acid backbone, nucleic acid sugar, or nucleic acid base modifications.

The term "biodegradable" as used herein, refers to degradation in a biological system, for example, enzymatic degradation or chemical degradation.

The term "biologically active molecule" as used herein refers to compounds or molecules that are capable of eliciting or modifying a biological response in a system. Non-limiting examples of biologically active siNA molecules either alone or in combination with other molecules contemplated by the instant invention include therapeutically active molecules such as antibodies, cholesterol, hormones, antivirals, peptides, proteins, chemotherapeutics, small molecules, vitamins, co-factors, nucleosides, nucleotides, oligonucleotides, enzymatic nucleic acids, antisense nucleic acids, triplex forming oligonucleotides, 2,5-A chimeras, siNA, dsRNA, allozymes, aptamers, decoys and analogs thereof. Biologically active molecules of the invention also include molecules capable of modulating the pharmacokinetics and/or pharmacodynamics of other biologically active molecules, for example, lipids and polymers such as polyamines, polyamides, polyethylene glycol and other polyethers.

The term "phospholipid" as used herein, refers to a hydrophobic molecule comprising at least one phosphorus group. For example, a phospholipid can comprise a phosphorus-containing group and saturated or unsaturated alkyl group, optionally substituted with OH, COOH, oxo, amine, or substituted or unsubstituted aryl groups.

Therapeutic nucleic acid molecules (*e.g.,* siNA molecules) delivered exogenously optimally are stable within cells until reverse transcription of the RNA has been modulated long enough to reduce the levels of the RNA transcript. The nucleic acid molecules are resistant to nucleases in order to function as effective intracellular therapeutic agents. Improvements in the chemical synthesis of nucleic acid molecules described in the instant invention and in the art have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

In yet another embodiment, siNA molecules having chemical modifications that maintain or enhance enzymatic activity of proteins involved in RNAi are provided. Such nucleic acids are also generally more resistant to nucleases than unmodified nucleic acids. Thus, *in vitro* and/or *in vivo* the activity should not be significantly lowered.

Use of the nucleic acid-based molecules of the invention will lead to better treatments by affording the possibility of combination therapies (*e.g.,* multiple siNA molecules targeted to different genes; nucleic acid molecules coupled with known small molecule modulators; or intermittent treatment with combinations of molecules, including different motifs and/or other chemical or biological molecules). The treatment of subjects with siNA molecules can also include combinations of different types of nucleic acid molecules, such as enzymatic nucleic acid molecules (ribozymes), allozymes, antisense, 2,5-A oligoadenylate, decoys, and aptamers.

In another aspect a siNA molecule of the invention comprises one or more 5' and/or a 3'- cap structure, for example, on only the sense siNA strand, the antisense siNA strand, or both siNA strands.

By "cap structure" is meant chemical modifications, which have been incorporated at either terminus of the oligonucleotide (see, for example, Adamic *et al.,* U.S. Pat. No. 5,998,203, incorporated by reference herein). These terminal modifications protect the nucleic acid molecule from exonuclease degradation, and may help in delivery and/or

localization within a cell. The cap may be present at the 5'-terminus (5'-cap) or at the 3'-terminal (3'-cap) or may be present on both termini. In non-limiting examples, the 5'-cap includes, but is not limited to, glyceryl, inverted deoxy abasic residue (moiety); 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide, 4'-thio nucleotide; carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; *threo*-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate moiety. Non-limiting examples of cap moieties are shown in Figure 10.

Non-limiting examples of the 3'-cap include, but are not limited to, glyceryl, inverted deoxy abasic residue (moiety), 4', 5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate; 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; *threo*-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties (for more details see Beaucage and Iyer, 1993, *Tetrahedron* 49, 1925; incorporated by reference herein).

By the term "non-nucleotide" is meant any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine and therefore lacks a base at the 1'-position.

An "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably, it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group can be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino, or SH. The term also includes alkenyl groups that are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably, it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2, halogen, $N(CH_3)_2$, amino, or SH. The term "alkyl" also includes alkynyl groups that have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably, it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino or SH.

Such alkyl groups can also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group that has at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

By "nucleotide" as used herein is as recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleotide sugar moiety. Nucleotides generally comprise a base, sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see, for example, Usman and McSwiggen, *supra*; Eckstein *et al.,* International PCT Publication No. WO 92/07065; Usman *et al.,* International PCT Publication No. WO 93/15187; Uhlman & Peyman, *supra,* all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach *et al.,* 1994, *Nucleic Acids Res.* 22, 2183. Some of the non-limiting examples of base modifications that can be introduced into nucleic acid molecules include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (*e.g.*, 5-methylcytidine), 5-alkyluridines (*e.g.*, ribothymidine), 5-halouridine (*e.g.*, 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (*e.g.* 6-methyluridine), propyne, and others (Burgin *et al.,* 1996, *Biochemistry,* 35, 14090; Uhlman & Peyman, *supra*). By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents.

In one embodiment, the invention features modified siNA molecules, with phosphate backbone modifications comprising one or more phosphorothioate, phosphorodithioate, methylphosphonate, phosphotriester, morpholino, amidate carbamate, carboxymethyl, acetamidate, polyamide, sulfonate, sulfonamide, sulfamate, formacetal, thioformacetal, and/or alkylsilyl, substitutions. For a review of oligonucleotide backbone modifications, see Hunziker and Leumann, 1995, *Nucleic Acid Analogues: Synthesis and Properties,* in *Modern Synthetic Methods,* VCH, 331-417, and Mesmaeker *et al.,* 1994, *Novel Backbone Replacements for Oligonucleotides,* in *Carbohydrate Modifications in Antisense Research,* ACS, 24-39.

By "abasic" is meant sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position, see for example Adamic *et al.,* U.S. Pat. No. 5,998,203.

By "unmodified nucleoside" is meant one of the bases adenine, cytosine, guanine, thymine, or uracil joined to the 1' carbon of β-D-ribo-furanose.

By "modified nucleoside" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base, sugar and/or phosphate. Non-limiting examples of modified nucleotides are shown by Formulae I-VII and/or other modifications described herein.

In connection with 2'-modified nucleotides as described for the present invention, by "amino" is meant 2'-NH_2 or 2'-*O*- NH_2, which can be modified or unmodified. Such modified groups are described, for example, in Eckstein *et al.,* U.S. Pat. No. 5,672,695 and Matulic-Adamic *et al.,* U.S. Pat. No. 6,248,878, which are both incorporated by reference in their entireties.

Various modifications to nucleic acid siNA structure can be made to enhance the utility of these molecules. Such modifications will enhance shelf-life, half-life *in vitro,* stability, and ease of introduction of such oligonucleotides to the target site, *e.g.,* to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

Administration of Nucleic Acid Molecules

A siNA molecule of the invention can be adapted for use to treat, prevent, inhibit, or reduce HCV infection, liver failure, hepatocellular carcinoma, cirrhosis and/or any other trait, disease or condition that is related to or will respond to the levels of HCV in a cell or tissue, alone or in combination with other therapies. In one embodiment, the siNA molecules of the invention and formulations or compositions thereof are administered to the liver as is generally known in the art (see for example Wen *et al.,* 2004, *World J Gastroenterol.,* 10, 244-9; Murao *et al.,* 2002, *Pharm Res.,* 19, 1808-14; Liu *et al.,* 2003, *Gene Ther.,* 10, 180-7; Hong *et al.,* 2003, *J Pharm Pharmacol.,* 54, 51-8; Herrmann *et al.,* 2004, *Arch Virol.,* 149, 1611-7; and Matsuno *et al.,* 2003, *Gene Ther.,* 10, 1559-66).

For example, a siNA molecule can comprise a delivery vehicle, including liposomes, for administration to a subject, carriers and diluents and their salts, and/or can be present in pharmaceutically acceptable formulations. Methods for the delivery of

nucleic acid molecules are described in Akhtar *et al.*, 1992, *Trends Cell Bio.*, 2, 139; *Delivery Strategies for Antisense Oligonucleotide Therapeutics*, ed. Akhtar, 1995, Maurer *et al.*, 1999, *Mol. Membr. Biol.*, 16, 129-140; Hofland and Huang, 1999, *Handb. Exp. Pharmacol.*, 137, 165-192; and Lee *et al.*, 2000, *ACS Symp. Ser.*, 752, 184-192, all of which are incorporated herein by reference. Beigelman *et al.*, U.S. Pat. No. 6,395,713 and Sullivan *et al.*, PCT WO 94/02595 further describe the general methods for delivery of nucleic acid molecules. These protocols can be utilized for the delivery of virtually any nucleic acid molecule. Nucleic acid molecules can be administered to cells by a variety of methods known to those of skill in the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as biodegradable polymers, hydrogels, cyclodextrins (see for example Gonzalez *et al.*, 1999, *Bioconjugate Chem.*, 10, 1068-1074; Wang *et al.*, International PCT publication Nos. WO 03/47518 and WO 03/46185), poly(lactic-co-glycolic)acid (PLGA) and PLCA microspheres (see for example US Patent 6,447,796 and US Patent Application Publication No. US 2002130430), biodegradable nanocapsules, and bioadhesive microspheres, or by proteinaceous vectors (O'Hare and Normand, International PCT Publication No. WO 00/53722). In another embodiment, the nucleic acid molecules of the invention can also be formulated or complexed with polyethyleneimine and derivatives thereof, such as polyethyleneimine-polyethyleneglycol-N-acetylgalactosamine (PEI-PEG-GAL) or polyethyleneimine-polyethyleneglycol-tri-N-acetylgalactosamine (PEI-PEG-triGAL) derivatives. In one embodiment, the nucleic acid molecules of the invention are formulated as described in United States Patent Application Publication No. 20030077829, incorporated by reference herein in its entirety.

In one embodiment, a siNA molecule of the invention is complexed with membrane disruptive agents such as those described in U.S. Patent Application Publication No. 20010007666, incorporated by reference herein in its entirety including the drawings. In another embodiment, the membrane disruptive agent or agents and the siNA molecule are also complexed with a cationic lipid or helper lipid molecule, such as those lipids described in U.S. Patent No. 6,235,310, incorporated by reference herein in its entirety including the drawings.

In one embodiment, a siNA molecule of the invention is complexed with delivery systems as described in U.S. Patent Application Publication No. 2003077829 and

International PCT Publication Nos. WO 00/03683 and WO 02/087541, all incorporated by reference herein in their entirety including the drawings.

In one embodiment, the siNA molecules of the invention and formulations or compositions thereof are administered directly or topically (e.g., locally) to the dermis or follicles as is generally known in the art (see for example Brand, 2001, *Curr. Opin. Mol. Ther.*, 3, 244-8; Regnier *et al.*, 1998, *J. Drug Target,* 5, 275-89; Kanikkannan, 2002, *BioDrugs,* 16, 339-47; Wraight *et al.*, 2001, *Pharmacol. Ther.*, 90, 89-104; Preat and Dujardin, 2001, STP PharmaSciences, 11, 57-68; and Vogt *et al.*, 2003, *Hautarzt.* 54, 692-8).

In one embodiment, delivery systems of the invention include, for example, aqueous and nonaqueous gels, creams, multiple emulsions, microemulsions, liposomes, ointments, aqueous and nonaqueous solutions, lotions, aerosols, hydrocarbon bases and powders, and can contain excipients such as solubilizers, permeation enhancers (e.g., fatty acids, fatty acid esters, fatty alcohols and amino acids), and hydrophilic polymers (e.g., polycarbophil and polyvinylpyrolidone). In one embodiment, the pharmaceutically acceptable carrier is a liposome or a transdermal enhancer. Examples of liposomes which can be used in this invention include the following: (1) CellFectin, 1:1.5 (M/M) liposome formulation of the cationic lipid N,NI,NII,NIII-tetramethyl-N,NI,NII,NIII-tetrapalmit-y-spermine and dioleoyl phosphatidylethanolamine (DOPE) (GIBCO BRL); (2) Cytofectin GSV, 2:1 (M/M) liposome formulation of a cationic lipid and DOPE (Glen Research); (3) DOTAP (N-[1-(2,3-dioleoyloxy)-N,N,N-tri-methyl-ammoniummethylsulfate) (Boehringer Manheim); and (4) Lipofectamine, 3:1 (M/M) liposome formulation of the polycationic lipid DOSPA and the neutral lipid DOPE (GIBCO BRL).

In one embodiment, delivery systems of the invention include patches, tablets, suppositories, pessaries, gels and creams, and can contain excipients such as solubilizers and enhancers (e.g., propylene glycol, bile salts and amino acids), and other vehicles (e.g., polyethylene glycol, fatty acid esters and derivatives, and hydrophilic polymers such as hydroxypropylmethylcellulose and hyaluronic acid).

In one embodiment, transdermal delivery systems of the invention include patches, tablets, suppositories, pessaries, gels and creams, and can contain excipients such as solubilizers and enhancers (e.g., propylene glycol, bile salts and amino acids), and other

vehicles (e.g., polyethylene glycol, fatty acid esters and derivatives, and hydrophilic polymers such as hydroxypropylmethylcellulose and hyaluronic acid).

In one embodiment, siNA molecules of the invention are formulated or complexed with polyethylenimine (e.g., linear or branched PEI) and/or polyethylenimine derivatives, including for example grafted PEIs such as galactose PEI, cholesterol PEI, antibody derivatized PEI, and polyethylene glycol PEI (PEG-PEI) derivatives thereof (see for example Ogris *et al.,* 2001, *AAPA PharmSci,* 3, 1-11; Furgeson et al., 2003, Bioconjugate Chem., 14, 840-847; Kunath et al., 2002, Phramaceutical Research, 19, 810-817; Choi et al., 2001, Bull. Korean Chem. Soc., 22, 46-52; Bettinger et al., 1999, Bioconjugate Chem., 10, 558-561; Peterson et al., 2002, Bioconjugate Chem., 13, 845-854; Erbacher et al., 1999, Journal of Gene Medicine Preprint, 1, 1-18; Godbey et al., 1999., PNAS USA, 96, 5177-5181; Godbey et al., 1999, Journal of Controlled Release, 60, 149-160; Diebold et al., 1999, Journal of Biological Chemistry, 274, 19087-19094; Thomas and Klibanov, 2002, PNAS USA, 99, 14640-14645; and Sagara, US 6,586,524, incorporated by reference herein.

In one embodiment, a siNA molecule of the invention comprises a bioconjugate, for example a nucleic acid conjugate as described in Vargeese et al., USSN 10/427,160, filed April 30, 2003; US 6,528,631; US 6,335,434; US 6, 235,886; US 6,153,737; US 5,214,136; US 5,138,045, all incorporated by reference herein.

Thus, the invention features a pharmaceutical composition comprising one or more nucleic acid(s) of the invention in an acceptable carrier, such as a stabilizer, buffer, and the like. The polynucleotides of the invention can be administered (*e.g.*, RNA, DNA or protein) and introduced to a subject by any standard means, with or without stabilizers, buffers, and the like, to form a pharmaceutical composition. When it is desired to use a liposome delivery mechanism, standard protocols for formation of liposomes can be followed. The compositions of the present invention can also be formulated and used as creams, gels, sprays, oils and other suitable compositions for topical, dermal, or transdermal administration as is known in the art.

The present invention also includes pharmaceutically acceptable formulations of the compounds described. These formulations include salts of the above compounds, *e.g.*, acid addition salts, for example, salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

A pharmacological composition or formulation refers to a composition or formulation in a form suitable for administration, *e.g.,* systemic or local administration, into a cell or subject, including for example a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should not prevent the composition or formulation from reaching a target cell (*i.e.*, a cell to which the negatively charged nucleic acid is desirable for delivery). For example, pharmacological compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms that prevent the composition or formulation from exerting its effect.

In one embodiment, siNA molecules of the invention are administered to a subject by systemic administration in a pharmaceutically acceptable composition or formulation. By "systemic administration" is meant *in vivo* systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes that lead to systemic absorption include, without limitation: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes exposes the siNA molecules of the invention to an accessible diseased tissue. The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compounds of the instant invention can potentially localize the drug, for example, in certain tissue types, such as the tissues of the reticular endothelial system (RES). A liposome formulation that can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is also useful. This approach can provide enhanced delivery of the drug to target cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of abnormal cells.

By "pharmaceutically acceptable formulation" or "pharmaceutically acceptable composition" is meant, a composition or formulation that allows for the effective distribution of the nucleic acid molecules of the instant invention in the physical location most suitable for their desired activity. Non-limiting examples of agents suitable for formulation with the nucleic acid molecules of the instant invention include: P-glycoprotein inhibitors (such as Pluronic P85),; biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery (Emerich, DF *et al*, 1999, *Cell Transplant*, 8, 47-58); and loaded nanoparticles, such as those made of

polybutylcyanoacrylate. Other non-limiting examples of delivery strategies for the nucleic acid molecules of the instant invention include material described in Boado *et al.*, 1998, *J. Pharm. Sci.*, 87, 1308-1315; Tyler *et al.*, 1999, *FEBS Lett.*, 421, 280-284; Pardridge *et al.*, 1995, *PNAS USA.*, 92, 5592-5596; Boado, 1995, *Adv. Drug Delivery Rev.*, 15, 73-107; Aldrian-Herrada *et al.*, 1998, *Nucleic Acids Res.*, 26, 4910-4916; and Tyler *et al.*, 1999, *PNAS USA.*, 96, 7053-7058.

The invention also features the use of a composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes) and nucleic acid molecules of the invention. These formulations offer a method for increasing the accumulation of drugs (e.g., siNA) in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug (Lasic *et al.* *Chem. Rev.* 1995, 95, 2601-2627; Ishiwata *et al.*, *Chem. Pharm. Bull.* 1995, 43, 1005-1011). Such liposomes have been shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic *et al.*, *Science* 1995, 267, 1275-1276; Oku *et al.*,1995, *Biochim. Biophys. Acta*, 1238, 86-90). The long-circulating liposomes enhance the pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu *et al.*, *J. Biol. Chem.* 1995, 42, 24864-24870; Choi *et al.*, International PCT Publication No. WO 96/10391; Ansell *et al.*, International PCT Publication No. WO 96/10390; Holland *et al.*, International PCT Publication No. WO 96/10392). Long-circulating liposomes are also likely to protect drugs from nuclease degradation to a greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen.

The present invention also includes compositions prepared for storage or administration that include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro edit. 1985), hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents can be provided. These include

sodium benzoate, sorbic acid and esters of *p*-hydroxybenzoic acid. In addition, antioxidants and suspending agents can be used.

A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors that those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent upon potency of the negatively charged polymer.

The nucleic acid molecules of the invention and formulations thereof can be administered orally, topically, parenterally, by inhalation or spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and/or vehicles. The term parenteral as used herein includes percutaneous, subcutaneous, intravascular (*e.g.*, intravenous), intramuscular, or intrathecal injection or infusion techniques and the like. In addition, there is provided a pharmaceutical formulation comprising a nucleic acid molecule of the invention and a pharmaceutically acceptable carrier. One or more nucleic acid molecules of the invention can be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants, and if desired other active ingredients. The pharmaceutical compositions containing nucleic acid molecules of the invention can be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more such sweetening agents, flavoring agents, coloring agents or preservative agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients can be, for example, inert diluents; such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and

disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia; and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques. In some cases such coatings can be prepared by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate can be employed.

Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in a mixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropyl-methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions can be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents can be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, can also be present.

Pharmaceutical compositions of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavoring agents.

Syrups and elixirs can be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol, glucose or sucrose. Such formulations can also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. The sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The nucleic acid molecules of the invention can also be administered in the form of suppositories, *e.g.*, for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the

rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

Nucleic acid molecules of the invention can be administered parenterally in a sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

Dosage levels of the order of from about 0.1 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 0.5 mg to about 7 g per subject per day). The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending upon the host treated and the particular mode of administration. Dosage unit forms generally contain between from about 1 mg to about 500 mg of an active ingredient.

It is understood that the specific dose level for any particular subject depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

For administration to non-human animals, the composition can also be added to the animal feed or drinking water. It can be convenient to formulate the animal feed and drinking water compositions so that the animal takes in a therapeutically appropriate quantity of the composition along with its diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water.

The nucleic acid molecules of the present invention can also be administered to a subject in combination with other therapeutic compounds to increase the overall therapeutic effect. The use of multiple compounds to treat an indication can increase the beneficial effects while reducing the presence of side effects.

In one embodiment, the invention comprises compositions suitable for administering nucleic acid molecules of the invention to specific cell types. For example, the asialoglycoprotein receptor (ASGPr) (Wu and Wu, 1987, *J. Biol. Chem.* 262, 4429-4432) is unique to hepatocytes and binds branched galactose-terminal

glycoproteins, such as asialoorosomucoid (ASOR). In another example, the folate receptor is overexpressed in many cancer cells. Binding of such glycoproteins, synthetic glycoconjugates, or folates to the receptor takes place with an affinity that strongly depends on the degree of branching of the oligosaccharide chain, for example, triatennary structures are bound with greater affinity than biatenarry or monoatennary chains (Baenziger and Fiete, 1980, *Cell,* 22, 611-620; Connolly *et al.,* 1982, *J. Biol. Chem.,* 257, 939-945). Lee and Lee, 1987, *Glycoconjugate J.,* 4, 317-328, obtained this high specificity through the use of N-acetyl-D-galactosamine as the carbohydrate moiety, which has higher affinity for the receptor, compared to galactose. This "clustering effect" has also been described for the binding and uptake of mannosyl-terminating glycoproteins or glycoconjugates (Ponpipom *et al.,* 1981, *J. Med. Chem.,* 24, 1388-1395). The use of galactose, galactosamine, or folate based conjugates to transport exogenous compounds across cell membranes can provide a targeted delivery approach to, for example, the treatment of liver disease, cancers of the liver, or other cancers. The use of bioconjugates can also provide a reduction in the required dose of therapeutic compounds required for treatment. Furthermore, therapeutic bioavailability, pharmacodynamics, and pharmacokinetic parameters can be modulated through the use of nucleic acid bioconjugates of the invention. Non-limiting examples of such bioconjugates are described in Vargeese *et al.,* USSN 10/201,394, filed August 13, 2001; and Matulic-Adamic *et al.,* USSN 60/362,016, filed March 6, 2002.

Alternatively, certain siNA molecules of the instant invention can be expressed within cells from eukaryotic promoters (*e.g.,* Izant and Weintraub, 1985, *Science,* 229, 345; McGarry and Lindquist, 1986, *Proc. Natl. Acad. Sci.,* USA 83, 399; Scanlon *et al.,* 1991, *Proc. Natl. Acad. Sci. USA,* 88, 10591-5; Kashani-Sabet *et al.,* 1992, *Antisense Res. Dev.,* 2, 3-15; Dropulic *et al.,* 1992, *J. Virol.,* 66, 1432-41; Weerasinghe *et al.,* 1991, *J. Virol.,* 65, 5531-4; Ojwang *et al.,* 1992, *Proc. Natl. Acad. Sci. USA,* 89, 10802-6; Chen *et al.,* 1992, *Nucleic Acids Res.,* 20, 4581-9; Sarver *et al.,* 1990 *Science,* 247, 1222-1225; Thompson *et al.,* 1995, *Nucleic Acids Res.,* 23, 2259; Good *et al.,* 1997, *Gene Therapy,* 4, 45. Those skilled in the art realize that any nucleic acid can be expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity of such nucleic acids can be augmented by their release from the primary transcript by a enzymatic nucleic acid (Draper *et al.,* PCT WO 93/23569, and Sullivan *et al.,* PCT WO 94/02595; Ohkawa *et al.,* 1992, *Nucleic Acids Symp. Ser.,* 27, 15-6; Taira *et al.,* 1991,

Nucleic Acids Res., 19, 5125-30; Ventura *et al.*, 1993, *Nucleic Acids Res.*, 21, 3249-55; Chowrira *et al.*, 1994, *J. Biol. Chem.*, 269, 25856.

In another aspect of the invention, RNA molecules of the present invention can be expressed from transcription units (see for example Couture *et al.*, 1996, *TIG.*, 12, 510) inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. In another embodiment, pol III based constructs are used to express nucleic acid molecules of the invention (see for example Thompson, U.S. Pats. Nos. 5,902,880 and 6,146,886). The recombinant vectors capable of expressing the siNA molecules can be delivered as described above, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of nucleic acid molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the siNA molecule interacts with the target mRNA and generates an RNAi response. Delivery of siNA molecule expressing vectors can be systemic, such as by intravenous or intra-muscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired target cell (for a review see Couture *et al.*, 1996, *TIG.*, 12, 510).

In one aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the instant invention. The expression vector can encode one or both strands of a siNA duplex, or a single self-complementary strand that self hybridizes into a siNA duplex. The nucleic acid sequences encoding the siNA molecules of the instant invention can be operably linked in a manner that allows expression of the siNA molecule (see for example Paul *et al.*, 2002, *Nature Biotechnology,* 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology,* 19, 497; Lee *et al.*, 2002, *Nature Biotechnology,* 19, 500; and Novina *et al.*, 2002, *Nature Medicine*, advance online publication doi:10.1038/nm725).

In another aspect, the invention features an expression vector comprising: a) a transcription initiation region (*e.g.*, eukaryotic pol I, II or III initiation region); b) a transcription termination region (*e.g.*, eukaryotic pol I, II or III termination region); and c) a nucleic acid sequence encoding at least one of the siNA molecules of the instant invention, wherein said sequence is operably linked to said initiation region and said

termination region in a manner that allows expression and/or delivery of the siNA molecule. The vector can optionally include an open reading frame (ORF) for a protein operably linked on the 5' side or the 3'-side of the sequence encoding the siNA of the invention; and/or an intron (intervening sequences).

Transcription of the siNA molecule sequences can be driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters are expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type depends on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990, *Proc. Natl. Acad. Sci. U S A*, 87, 6743-7; Gao and Huang 1993, *Nucleic Acids Res*., 21, 2867-72; Lieber *et al.*, 1993, *Methods Enzymol.*, 217, 47-66; Zhou *et al.*, 1990, *Mol. Cell. Biol*., 10, 4529-37). Several investigators have demonstrated that nucleic acid molecules expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet *et al.*, 1992, *Antisense Res. Dev.*, 2, 3-15; Ojwang *et al.*, 1992, *Proc. Natl. Acad. Sci. U S A*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res*., 20, 4581-9; Yu *et al.*, 1993, *Proc. Natl. Acad. Sci. U S A*, 90, 6340-4; L'Huillier *et al.*, 1992, *EMBO J.*, 11, 4411-8; Lisziewicz *et al.*, 1993, *Proc. Natl. Acad. Sci. U. S. A*, 90, 8000-4; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; Sullenger & Cech, 1993, *Science*, 262, 1566). More specifically, transcription units such as the ones derived from genes encoding U6 small nuclear (snRNA), transfer RNA (tRNA) and adenovirus VA RNA are useful in generating high concentrations of desired RNA molecules such as siNA in cells (Thompson *et al., supra*; Couture and Stinchcomb, *1996, supra*; Noonberg *et al.*, 1994, *Nucleic Acid Res*., 22, 2830; Noonberg *et al.*, U.S. Pat. No. 5,624,803; Good *et al.*, 1997, *Gene Ther.*, 4, 45; Beigelman *et al.*, International PCT Publication No. WO 96/18736. The above siNA transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review see Couture and Stinchcomb, *1996, supra)*.

In another aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one of the siNA molecules of the invention in a manner

that allows expression of that siNA molecule. The expression vector comprises in one embodiment; a) a transcription initiation region; b) a transcription termination region; and c) a nucleic acid sequence encoding at least one strand of the siNA molecule, wherein the sequence is operably linked to the initiation region and the termination region in a manner that allows expression and/or delivery of the siNA molecule.

In another embodiment the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an open reading frame; and d) a nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame and wherein the sequence is operably linked to the initiation region, the open reading frame and the termination region in a manner that allows expression and/or delivery of the siNA molecule. In yet another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; and d) a nucleic acid sequence encoding at least one siNA molecule, wherein the sequence is operably linked to the initiation region, the intron and the termination region in a manner which allows expression and/or delivery of the nucleic acid molecule.

In another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) an open reading frame; and e) a nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame and wherein the sequence is operably linked to the initiation region, the intron, the open reading frame and the termination region in a manner which allows expression and/or delivery of the siNA molecule.

HCV biology and biochemistry

In 1989, the Hepatitis C Virus (HCV) was determined to be an RNA virus and was identified as the causative agent of most non-A non-B viral Hepatitis (Choo *et al.,* 1989, *Science*, 244, 359-362). Unlike retroviruses such as HIV, HCV does not go though a DNA replication phase and no integrated forms of the viral genome into the host chromosome have been detected (Houghton *et al.,* 1991, *Hepatology,* 14, 381-388). Rather, replication of the coding (plus) strand is mediated by the production of a replicative (minus) strand leading to the generation of several copies of plus strand HCV RNA. The genome consists of a single, large, open-reading frame that is translated into

a polyprotein (Kato *et al.,* 1991, *FEBS Letters*, 280: 325-328). This polyprotein subsequently undergoes post-translational cleavage, producing several viral proteins (Leinbach *et al.,* 1994, *Virology,* 204:163-169).

Examination of the 9.5-kilobase genome of HCV has demonstrated that the viral nucleic acid can mutate at a high rate (Smith *et al.,* 1997 *Mol. Evol.* 45, 238-246). This rate of mutation has led to the evolution of several distinct genotypes of HCV that share approximately 70% sequence identity (Simmonds *et al.,* 1994, *J. Gen. Virol.* 75, 1053-1061). It is important to note that these sequences are evolutionarily quite distant. For example, the genetic identity between humans and primates such as the chimpanzee is approximately 98%. In addition, it has been demonstrated that an HCV infection in an individual patient is composed of several distinct and evolving quasispecies that have 98% identity at the RNA level. Thus, the HCV genome is hypervariable and continuously changing. Although the HCV genome is hypervariable, there are 3 regions of the genome that are highly conserved. These conserved sequences occur in the 5' and 3' non-coding regions as well as the 5'-end of the core protein coding region and are thought to be vital for HCV RNA replication as well as translation of the HCV polyprotein. Thus, therapeutic agents that target these conserved HCV genomic regions may have a significant impact over a wide range of HCV genotypes. Moreover, it is unlikely that drug resistance will occur with enzymatic nucleic acids specific to conserved regions of the HCV genome. In contrast, therapeutic modalities that target inhibition of enzymes such as the viral proteases or helicase are likely to result in the selection for drug resistant strains since the RNA for these viral encoded enzymes is located in the hypervariable portion of the HCV genome.

After initial exposure to HCV, a patient experiences a transient rise in liver enzymes, which indicates that inflammatory processes are occurring (Alter *et al.,* IN: Seeff LB, Lewis JH, eds. *Current Perspectives in Hepatology.* New York: Plenum Medical Book Co; 1989:83-89). This elevation in liver enzymes occurs at least 4 weeks after the initial exposure and may last for up to two months (Farci *et al.,* 1991, *New England Journal of Medicine.* 325, 98-104). Prior to the rise in liver enzymes, it is possible to detect HCV RNA in the patient's serum using RT-PCR analysis (Takahashi *et al.,* 1993, *American Journal of Gastroenterology.* 88, 240-243). This stage of the disease is called the acute stage and usually goes undetected since 75% of patients with acute

viral hepatitis from HCV infection are asymptomatic. The remaining 25% of these patients develop jaundice or other symptoms of hepatitis.

Although acute HCV infection is a benign disease, as many as 80% of acute HCV patients progress to chronic liver disease as evidenced by persistent elevation of serum alanine aminotransferase (ALT) levels and by continual presence of circulating HCV RNA (Sherlock, 1992, *Lancet*, 339, 802). The natural progression of chronic HCV infection over a 10 to 20 year period leads to cirrhosis in 20 to 50% of patients (Davis *et al.*, 1993, *Infectious Agents and Disease*, 2, 150, 154) and progression of HCV infection to hepatocellular carcinoma has been well documented (Liang *et al.*, 1993, *Hepatology*. 18, 1326-1333; Tong *et al.*, 1994, *Western Journal of Medicine*, 160, 133-138). There have been no studies that have determined sub-populations that are most likely to progress to cirrhosis and/or hepatocellular carcinoma, thus all patients have equal risk of progression.

It is important to note that the survival for patients diagnosed with hepatocellular carcinoma is only 0.9 to 12.8 months from initial diagnosis (Takahashi *et al.*, 1993, *American Journal of Gastroenterology*. 88, 240-243). Treatment of hepatocellular carcinoma with chemotherapeutic agents has not proven effective and only 10% of patients will benefit from surgery due to extensive tumor invasion of the liver (Trinchet *et al.*, 1994, *Presse Medicine*. 23, 831-833). Given the aggressive nature of primary hepatocellular carcinoma, the only viable treatment alternative to surgery is liver transplantation (Pichlmayr *et al.*, 1994, *Hepatology*. 20, 33S-40S).

Upon progression to cirrhosis, patients with chronic HCV infection present with clinical features, which are common to clinical cirrhosis regardless of the initial cause (D'Amico *et al.*, 1986, *Digestive Diseases and Sciences*. 31, 468-475). These clinical features may include: bleeding esophageal varices, ascites, jaundice, and encephalopathy (Zakim D, Boyer TD. Hepatology a textbook of liver disease. Second Edition Volume 1. 1990 W.B. Saunders Company. Philadelphia). In the early stages of cirrhosis, patients are classified as compensated, the stage at which the patient's liver is still able to detoxify metabolites in the blood-stream although liver tissue damage has occurred. In addition, most patients with compensated liver disease are asymptomatic and the minority with symptoms report only minor symptoms, such as dyspepsia and weakness. In the later stages of cirrhosis, patients are classified as decompensated, the stage at which the ability

of the liver to detoxify metabolites in the bloodstream is diminished. It is at the decompensated stage that the clinical features described above present.

In 1986, D'Amico *et al.* described the clinical manifestations and survival rates in 1155 patients with both alcoholic and viral associated cirrhosis (D'Amico *supra*). Of the 1155 patients, 435 (37%) had compensated disease although 70% were asymptomatic at the beginning of the study. The remaining 720 patients (63%) had decompensated liver disease with 78% presenting with a history of ascites, 31% with jaundice, 17% had bleeding and 16% had encephalopathy. Hepatocellular carcinoma was observed in six (.5%) patients with compensated disease and in 30 (2.6%) patients with decompensated disease.

Over the course of six years, the patients with compensated cirrhosis developed clinical features of decompensated disease at a rate of 10% per year. In most cases, ascites was the first presentation of decompensation. In addition, hepatocellular carcinoma developed in 59 patients who initially presented with compensated disease by the end of the six-year study.

With respect to survival, the D'Amico study indicated that the five-year survival rate for all patients in the study was only 40%. The six-year survival rate for the patients who initially had compensated cirrhosis was 54% while the six-year survival rate for patients who initially presented with decompensated disease was only 21%. There were no significant differences in the survival rates between the patients who had alcoholic cirrhosis and the patients with viral related cirrhosis. The major causes of death for the patients in the D'Amico study were liver failure in 49%; hepatocellular carcinoma in 22%; and bleeding in 13% (D'Amico *supra*).

Chronic Hepatitis C is a slowly progressing inflammatory disease of the liver, mediated by a virus (HCV) that can lead to cirrhosis, liver failure and/or hepatocellular carcinoma over a period of 10 to 20 years. In the US, it is estimated that infection with HCV accounts for 50,000 new cases of acute hepatitis in the United States each year (NIH Consensus Development Conference Statement on Management of Hepatitis C March 1997). The prevalence of HCV in the United States is estimated at 1.8% and the CDC places the number of chronically infected Americans at approximately 4.5 million people. The CDC also estimates that up to 10,000 deaths per year are caused by chronic HCV infection.

Numerous well controlled clinical trials using interferon (IFN-alpha) in the treatment of chronic HCV infection have demonstrated that treatment three times a week results in lowering of serum ALT values in approximately 50% (40% - 70%) of patients by the end of 6 months of therapy (Davis *et al.*, 1989, *New England Journal of Medicine,* 321, 1501-1506; Marcellin *et al.*, 1991, *Hepatology,* 13, 393-397; Tong *et al.*, 1997, *Hepatology,* 26, 747-754; Tong *et al.*, 1997, *Hepatology,* 26, 1640-1645). However, following cessation of interferon treatment, approximately 50% of the responding patients relapsed, resulting in a "durable" response rate as assessed by normalization of serum ALT concentrations of approximately 20 - 25%.

Direct measurement of HCV RNA is possible through use of either the branched-DNA or Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) analysis. In general, RT-PCR methodology is more sensitive and leads to a more accurate assessment of the clinical course (Tong *et al., supra*). Studies that have examined six months of type 1 interferon therapy using changes in HCV RNA values as a clinical endpoint have demonstrated that up to 35% of patients have a loss of HCV RNA by the end of therapy (Marcellin *et al., supra*). However, as with the ALT endpoint, about 50% of the patients relapse within six months following cessation of therapy, resulting in a durable virologic response of only 12% (Marcellin *et al., supra*). Studies that have examined 48 weeks of therapy have demonstrated that the sustained virological response is up to 25% (NIH consensus statement: 1997). Thus, standard of care for treatment of chronic HCV infection with type 1 interferon is now 48 weeks of therapy using changes in HCV RNA concentrations as the primary assessment of efficacy (Hoofnagle *et al.*, 1997, *New England Journal of Medicine,* 336, 347-356).

Side effects resulting from treatment with type 1 interferons can be divided into four general categories, which include: (1) Influenza-like symptoms; (2) Neuropsychiatric; (3) Laboratory abnormalities; and (4) Miscellaneous (Dusheiko *et al.*, 1994, Journal of Viral Hepatitis, 1, 3-5). Examples of influenza-like symptoms include fatigue, fever, myalgia, malaise, appetite loss, tachycardia, rigors, headache, and arthralgias. The influenza-like symptoms are usually short-lived and tend to abate after the first four weeks of dosing (Dushieko *et al., supra*). Neuropsychiatric side effects include irritability, apathy, mood changes, insomnia, cognitive changes, and depression. The most important of these neuropsychiatric side effects is depression and patients who have a history of depression should not be given type 1 interferon. Laboratory

abnormalities include reduction in myeloid cells, including granulocytes, platelets and to a lesser extent red blood cells. These changes in blood cell counts rarely lead to any significant clinical sequellae (Dushieko *et al., supra*). In addition, increases in triglyceride concentrations and elevations in serum alanine and aspartate aminotransferase concentration have been observed. Finally, thyroid abnormalities have been reported. These thyroid abnormalities are usually reversible after cessation of interferon therapy and can be controlled with appropriate medication while on therapy. Miscellaneous side effects include nausea, diarrhea, abdominal and back pain, pruritus, alopecia, and rhinorrhea. In general, most side effects will abate after 4 to 8 weeks of therapy (Dushieko *et al., supra*).

The use of small interfering nucleic acid molecules targeting HCV genes and cellular/host gene targets associated with the HIV life cycle therefore provides a class of novel therapeutic agents that can be used in the treatment and diagnosis of HCV infection, liver failure, hepatocellular carcinoma, cirrhosis or any other disease or condition that responds to modulation (e.g., inhibition) of HCV genes in a subject or organism.

Examples:

The following are non-limiting examples showing the selection, isolation, synthesis and activity of nucleic acids of the instant invention.

Example 1: Tandem synthesis of siNA constructs

Exemplary siNA molecules of the invention are synthesized in tandem using a cleavable linker, for example, a succinyl-based linker. Tandem synthesis as described herein is followed by a one-step purification process that provides RNAi molecules in high yield. This approach is highly amenable to siNA synthesis in support of high throughput RNAi screening, and can be readily adapted to multi-column or multi-well synthesis platforms.

After completing a tandem synthesis of a siNA oligo and its complement in which the 5'-terminal dimethoxytrityl (5'-O-DMT) group remains intact (trityl on synthesis), the oligonucleotides are deprotected as described above. Following deprotection, the siNA sequence strands are allowed to spontaneously hybridize. This hybridization yields a

duplex in which one strand has retained the 5'-O-DMT group while the complementary strand comprises a terminal 5'-hydroxyl. The newly formed duplex behaves as a single molecule during routine solid-phase extraction purification (Trityl-On purification) even though only one molecule has a dimethoxytrityl group. Because the strands form a stable duplex, this dimethoxytrityl group (or an equivalent group, such as other trityl groups or other hydrophobic moieties) is all that is required to purify the pair of oligos, for example, by using a C18 cartridge.

Standard phosphoramidite synthesis chemistry is used up to the point of introducing a tandem linker, such as an inverted deoxy abasic succinate or glyceryl succinate linker (see **Figure 1**) or an equivalent cleavable linker. A non-limiting example of linker coupling conditions that can be used includes a hindered base such as diisopropylethylamine (DIPA) and/or DMAP in the presence of an activator reagent such as Bromotripyrrolidinophosphoniumhexaflurorophosphate (PyBrOP). After the linker is coupled, standard synthesis chemistry is utilized to complete synthesis of the second sequence leaving the terminal the 5'-O-DMT intact. Following synthesis, the resulting oligonucleotide is deprotected according to the procedures described herein and quenched with a suitable buffer, for example with 50mM NaOAc or 1.5M $NH_4H_2CO_3$.

Purification of the siNA duplex can be readily accomplished using solid phase extraction, for example, using a Waters C18 SepPak 1g cartridge conditioned with 1 column volume (CV) of acetonitrile, 2 CV H2O, and 2 CV 50mM NaOAc. The sample is loaded and then washed with 1 CV H2O or 50mM NaOAc. Failure sequences are eluted with 1 CV 14% ACN (Aqueous with 50mM NaOAc and 50mM NaCl). The column is then washed, for example with 1 CV H2O followed by on-column detritylation, for example by passing 1 CV of 1% aqueous trifluoroacetic acid (TFA) over the column, then adding a second CV of 1% aqueous TFA to the column and allowing to stand for approximately 10 minutes. The remaining TFA solution is removed and the column washed with H20 followed by 1 CV 1M NaCl and additional H2O. The siNA duplex product is then eluted, for example, using 1 CV 20% aqueous CAN.

Figure 2 provides an example of MALDI-TOF mass spectrometry analysis of a purified siNA construct in which each peak corresponds to the calculated mass of an individual siNA strand of the siNA duplex. The same purified siNA provides three

peaks when analyzed by capillary gel electrophoresis (CGE), one peak presumably corresponding to the duplex siNA, and two peaks presumably corresponding to the separate siNA sequence strands. Ion exchange HPLC analysis of the same siNA contract only shows a single peak. Testing of the purified siNA construct using a luciferase reporter assay described below demonstrated the same RNAi activity compared to siNA constructs generated from separately synthesized oligonucleotide sequence strands.

Example 2: Identification of potential siNA target sites in any RNA sequence

The sequence of an RNA target of interest, such as a viral or human mRNA transcript, is screened for target sites, for example by using a computer folding algorithm. In a non-limiting example, the sequence of a gene or RNA gene transcript derived from a database, such as Genbank, is used to generate siNA targets having complementarity to the target. Such sequences can be obtained from a database, or can be determined experimentally as known in the art. Target sites that are known, for example, those target sites determined to be effective target sites based on studies with other nucleic acid molecules, for example ribozymes or antisense, or those targets known to be associated with a disease or condition such as those sites containing mutations or deletions, can be used to design siNA molecules targeting those sites. Various parameters can be used to determine which sites are the most suitable target sites within the target RNA sequence. These parameters include but are not limited to secondary or tertiary RNA structure, the nucleotide base composition of the target sequence, the degree of homology between various regions of the target sequence, or the relative position of the target sequence within the RNA transcript. Based on these determinations, any number of target sites within the RNA transcript can be chosen to screen siNA molecules for efficacy, for example by using *in vitro* RNA cleavage assays, cell culture, or animal models. In a non-limiting example, anywhere from 1 to 1000 target sites are chosen within the transcript based on the size of the siNA construct to be used. High throughput screening assays can be developed for screening siNA molecules using methods known in the art, such as with multi-well or multi-plate assays to determine efficient reduction in target gene expression.

Example 3: Selection of siNA molecule target sites in a RNA

The following non-limiting steps can be used to carry out the selection of siNAs targeting a given gene sequence or transcript.

1. The target sequence is parsed *in silico* into a list of all fragments or subsequences of a particular length, for example 23 nucleotide fragments, contained within the target sequence. This step is typically carried out using a custom Perl script, but commercial sequence analysis programs such as Oligo, MacVector, or the GCG Wisconsin Package can be employed as well.

2. In some instances the siNAs correspond to more than one target sequence; such would be the case for example in targeting different transcripts of the same gene, targeting different transcripts of more than one gene, or for targeting both the human gene and an animal homolog. In this case, a subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find matching sequences in each list. The subsequences are then ranked according to the number of target sequences that contain the given subsequence; the goal is to find subsequences that are present in most or all of the target sequences. Alternately, the ranking can identify subsequences that are unique to a target sequence, such as a mutant target sequence. Such an approach would enable the use of siNA to target specifically the mutant sequence and not effect the expression of the normal sequence.

3. In some instances the siNA subsequences are absent in one or more sequences while present in the desired target sequence; such would be the case if the siNA targets a gene with a paralogous family member that is to remain untargeted. As in case 2 above, a subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find sequences that are present in the target gene but are absent in the untargeted paralog.

4. The ranked siNA subsequences can be further analyzed and ranked according to GC content. A preference can be given to sites containing 30-70% GC, with a further preference to sites containing 40-60% GC.

5. The ranked siNA subsequences can be further analyzed and ranked according to self-folding and internal hairpins. Weaker internal folds are preferred; strong hairpin structures are to be avoided.

6. The ranked siNA subsequences can be further analyzed and ranked according to whether they have runs of GGG or CCC in the sequence. GGG (or even more Gs) in either strand can make oligonucleotide synthesis problematic and can potentially

interfere with RNAi activity, so it is avoided whenever better sequences are available. CCC is searched in the target strand because that will place GGG in the antisense strand.

7. The ranked siNA subsequences can be further analyzed and ranked according to whether they have the dinucleotide UU (uridine dinucleotide) on the 3'-end of the sequence, and/or AA on the 5'-end of the sequence (to yield 3' UU on the antisense sequence). These sequences allow one to design siNA molecules with terminal TT thymidine dinucleotides.

8. Four or five target sites are chosen from the ranked list of subsequences as described above. For example, in subsequences having 23 nucleotides, the right 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the upper (sense) strand of the siNA duplex, while the reverse complement of the left 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the lower (antisense) strand of the siNA duplex (see **Tables II and III**). If terminal TT residues are desired for the sequence (as described in paragraph 7), then the two 3' terminal nucleotides of both the sense and antisense strands are replaced by TT prior to synthesizing the oligos.

9. The siNA molecules are screened in an *in vitro*, cell culture or animal model system to identify the most active siNA molecule or the most preferred target site within the target RNA sequence.

10. Other design considerations can be used when selecting target nucleic acid sequences, see, for example, Reynolds *et al.*, 2004, *Nature Biotechnology Advanced Online Publication*, 1 February 2004, doi:10.1038/nbt936 and Ui-Tei et al., 2004, Nucleic Acids Research, 32, doi:10.1093/nar/gkh247.

In an alternate approach, a pool of siNA constructs specific to a HCV target sequence is used to screen for target sites in cells expressing HCV RNA, such as human hepatoma (Huh7) cells (see for example Randall *et al.*, 2003, *PNAS USA*, 100, 235-240). The general strategy used in this approach is shown in **Figure 9**. A non-limiting example of such is a pool comprising sequences having any of SEQ ID NOS 1-2027. Cells expressing HCV are transfected with the pool of siNA constructs and cells that demonstrate a phenotype associated with HCV inhibition are sorted. The pool of siNA

constructs can be expressed from transcription cassettes inserted into appropriate vectors (see for example **Figure 7** and **Figure 8**). The siNA from cells demonstrating a positive phenotypic change (e.g., decreased HCV mRNA levels or decreased HCV protein expression), are sequenced to determine the most suitable target site(s) within the target HCV RNA sequence.

Example 4: HCV targeted siNA design

siNA target sites were chosen by analyzing sequences of the HCV RNA target and optionally prioritizing the target sites on the basis of folding (structure of any given sequence analyzed to determine siNA accessibility to the target), by using a library of siNA molecules as described in Example 3, or alternately by using an *in vitro* siNA system as described in Example 6 herein. siNA molecules were designed that could bind each target and are optionally individually analyzed by computer folding to assess whether the siNA molecule can interact with the target sequence. Varying the length of the siNA molecules can be chosen to optimize activity. Generally, a sufficient number of complementary nucleotide bases are chosen to bind to, or otherwise interact with, the target RNA, but the degree of complementarity can be modulated to accommodate siNA duplexes or varying length or base composition. By using such methodologies, siNA molecules can be designed to target sites within any known RNA sequence, for example those RNA sequences corresponding to the any gene transcript.

Chemically modified siNA constructs are designed to provide nuclease stability for systemic administration in vivo and/or improved pharmacokinetic, localization, and delivery properties while preserving the ability to mediate RNAi activity. Chemical modifications as described herein are introduced synthetically using synthetic methods described herein and those generally known in the art. The synthetic siNA constructs are then assayed for nuclease stability in serum and/or cellular/tissue extracts (e.g. liver extracts). The synthetic siNA constructs are also tested in parallel for RNAi activity using an appropriate assay, such as a luciferase reporter assay as described herein or another suitable assay that can quantity RNAi activity. Synthetic siNA constructs that possess both nuclease stability and RNAi activity can be further modified and re-evaluated in stability and activity assays. The chemical modifications of the stabilized active siNA constructs can then be applied to any siNA sequence targeting any chosen

RNA and used, for example, in target screening assays to pick lead siNA compounds for therapeutic development (see for example **Figure 11**).

Example 5: Chemical Synthesis and Purification of siNA

siNA molecules can be designed to interact with various sites in the RNA message, for example, target sequences within the RNA sequences described herein. The sequence of one strand of the siNA molecule(s) is complementary to the target site sequences described above. The siNA molecules can be chemically synthesized using methods described herein. Inactive siNA molecules that are used as control sequences can be synthesized by scrambling the sequence of the siNA molecules such that it is not complementary to the target sequence. Generally, siNA constructs can by synthesized using solid phase oligonucleotide synthesis methods as described herein (see for example Usman *et al.,* US Patent Nos. 5,804,683; 5,831,071; 5,998,203; 6,117,657; 6,353,098; 6,362,323; 6,437,117; 6,469,158; Scaringe *et al.,* US Patent Nos. 6,111,086; 6,008,400; 6,111,086 all incorporated by reference herein in their entirety).

In a non-limiting example, RNA oligonucleotides are synthesized in a stepwise fashion using the phosphoramidite chemistry as is known in the art. Standard phosphoramidite chemistry involves the use of nucleosides comprising any of 5'-O-dimethoxytrityl, 2'-O-tert-butyldimethylsilyl, 3'-O-2-Cyanoethyl N,N-diisopropylphos-phoroamidite groups, and exocyclic amine protecting groups (e.g. N6-benzoyl adenosine, N4 acetyl cytidine, and N2-isobutyryl guanosine). Alternately, 2′-O-Silyl Ethers can be used in conjunction with acid-labile 2′-O-orthoester protecting groups in the synthesis of RNA as described by Scaringe *supra.* Differing 2' chemistries can require different protecting groups, for example 2'-deoxy-2'-amino nucleosides can utilize N-phthaloyl protection as described by Usman *et al.,* US Patent 5,631,360, incorporated by reference herein in its entirety).

During solid phase synthesis, each nucleotide is added sequentially (3′- to 5′-direction) to the solid support-bound oligonucleotide. The first nucleoside at the 3′-end of the chain is covalently attached to a solid support (e.g., controlled pore glass or polystyrene) using various linkers. The nucleotide precursor, a ribonucleoside phosphoramidite, and activator are combined resulting in the coupling of the second nucleoside phosphoramidite onto the 5′-end of the first nucleoside. The support is then washed and any unreacted 5′-hydroxyl groups are capped with a capping reagent such as

acetic anhydride to yield inactive 5′-acetyl moieties. The trivalent phosphorus linkage is then oxidized to a more stable phosphate linkage. At the end of the nucleotide addition cycle, the 5′-O-protecting group is cleaved under suitable conditions (e.g., acidic conditions for trityl-based groups and Fluoride for silyl-based groups). The cycle is repeated for each subsequent nucleotide.

Modification of synthesis conditions can be used to optimize coupling efficiency, for example by using differing coupling times, differing reagent/phosphoramidite concentrations, differing contact times, differing solid supports and solid support linker chemistries depending on the particular chemical composition of the siNA to be synthesized. Deprotection and purification of the siNA can be performed as is generally described in Usman *et al.*, US 5,831,071, US 6,353,098, US 6,437,117, and Bellon *et al.*, US 6,054,576, US 6,162,909, US 6,303,773, or Scaringe *supra,* incorporated by reference herein in their entireties. Additionally, deprotection conditions can be modified to provide the best possible yield and purity of siNA constructs. For example, applicant has observed that oligonucleotides comprising 2'-deoxy-2'-fluoro nucleotides can degrade under inappropriate deprotection conditions. Such oligonucleotides are deprotected using aqueous methylamine at about 35°C for 30 minutes. If the 2'-deoxy-2'-fluoro containing oligonucleotide also comprises ribonucleotides, after deprotection with aqueous methylamine at about 35°C for 30 minutes, TEA-HF is added and the reaction maintained at about 65°C for an additional 15 minutes.

Example 6: RNAi *in vitro* assay to assess siNA activity

An *in vitro* assay that recapitulates RNAi in a cell-free system is used to evaluate siNA constructs targeting HCV RNA targets. The assay comprises the system described by Tuschl *et al.,* 1999, *Genes and Development*, 13, 3191-3197 and Zamore *et al.,* 2000, *Cell*, 101, 25-33 adapted for use with HCV target RNA. A Drosophila extract derived from syncytial blastoderm is used to reconstitute RNAi activity *in vitro*. Target RNA is generated via *in vitro* transcription from an appropriate HCV expressing plasmid using T7 RNA polymerase or via chemical synthesis as described herein. Sense and antisense siNA strands (for example 20 uM each) are annealed by incubation in buffer (such as 100 mM potassium acetate, 30 mM HEPES-KOH, pH 7.4, 2 mM magnesium acetate) for 1 minute at 90°C followed by 1 hour at 37°C , then diluted in lysis buffer (for example 100 mM potassium acetate, 30 mM HEPES-KOH at pH 7.4, 2mM magnesium acetate).

Annealing can be monitored by gel electrophoresis on an agarose gel in TBE buffer and stained with ethidium bromide. The Drosophila lysate is prepared using zero to two-hour-old embryos from Oregon R flies collected on yeasted molasses agar that are dechorionated and lysed. The lysate is centrifuged and the supernatant isolated. The assay comprises a reaction mixture containing 50% lysate [vol/vol], RNA (10-50 pM final concentration), and 10% [vol/vol] lysis buffer containing siNA (10 nM final concentration). The reaction mixture also contains 10 mM creatine phosphate, 10 ug/ml creatine phosphokinase, 100 um GTP, 100 uM UTP, 100 uM CTP, 500 uM ATP, 5 mM DTT, 0.1 U/uL RNasin (Promega), and 100 uM of each amino acid. The final concentration of potassium acetate is adjusted to 100 mM. The reactions are pre-assembled on ice and preincubated at 25° C for 10 minutes before adding RNA, then incubated at 25° C for an additional 60 minutes. Reactions are quenched with 4 volumes of 1.25 x Passive Lysis Buffer (Promega). Target RNA cleavage is assayed by RT-PCR analysis or other methods known in the art and are compared to control reactions in which siNA is omitted from the reaction.

Alternately, internally-labeled target RNA for the assay is prepared by *in vitro* transcription in the presence of [alpha-^{32}P] CTP, passed over a G50 Sephadex column by spin chromatography and used as target RNA without further purification. Optionally, target RNA is 5'-^{32}P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed as described above and target RNA and the specific RNA cleavage products generated by RNAi are visualized on an autoradiograph of a gel. The percentage of cleavage is determined by PHOSPHOR IMAGER® (autoradiography) quantitation of bands representing intact control RNA or RNA from control reactions without siNA and the cleavage products generated by the assay.

In one embodiment, this assay is used to determine target sites in the HCV RNA target for siNA mediated RNAi cleavage, wherein a plurality of siNA constructs are screened for RNAi mediated cleavage of the HCV RNA target, for example, by analyzing the assay reaction by electrophoresis of labeled target RNA, or by northern blotting, as well as by other methodology well known in the art.

Example 7: Nucleic acid inhibition of HCV target RNA

siNA molecules targeted to the human HCV RNA are designed and synthesized as described above. These nucleic acid molecules can be tested for cleavage activity *in vivo*, for example, using the following procedure. The target sequences and the nucleotide location within the HCV RNA are given in **Tables II and III**.

Two formats are used to test the efficacy of siNAs targeting HCV. First, the reagents are tested in cell culture using, for example, human hepatoma (Huh7) cells, to determine the extent of RNA and protein inhibition. siNA reagents (*e.g.*; see **Tables II and III**) are selected against the HCV target as described herein. RNA inhibition is measured after delivery of these reagents by a suitable transfection agent to, for example, cultured epidermal keratinocytes. Relative amounts of target RNA are measured versus actin using real-time PCR monitoring of amplification (*eg.*, ABI 7700 TAQMAN®). A comparison is made to a mixture of oligonucleotide sequences made to unrelated targets or to a randomized siNA control with the same overall length and chemistry, but randomly substituted at each position. Primary and secondary lead reagents are chosen for the target and optimization performed. After an optimal transfection agent concentration is chosen, a RNA time-course of inhibition is performed with the lead siNA molecule. In addition, a cell-plating format can be used to determine RNA inhibition.

In addition, a cell-plating format can be used to determine RNA inhibition. A non-limiting example of a multiple target screen to assay siNA mediated inhibition of HCV RNA is shown in **Figure 28**. siNA constructs (Table III) were transfected at 25 nM into Huh7 cells and HCV RNA quantitated compared to untreated cells ("cells" column in the figure) and cells transfected with lipofectamine ("LFA2K" column in the figure). As shown in **Figure 28**, several siNA constructs show significant inhibition of HCV RNA expression in the Huh7 replicon system. This system is described in Rice *et al.*, US 5,874,565 and US 6,127,116, both incorporated by reference herein.

<u>Delivery of siNA to Cells</u>

Huh7b cells stably transfected with the HCV subgenomic replicon Clone A or Ava.5 are seeded, for example, at 8.5×10^3 cells per well of a 96-well platein DMEM(Gibco) the day before transfection. siNA (final concentration, for example 25nM) and cationic lipid Lipofectamine2000 (e.g., final concentration0.5ul/well) are

complexed in Optimem (Gibco) at 37°C for 20 minutes inpolypropelyne microtubes. Following vortexing, the complexed siNA is added to each well and incubated for 24-72 hours.

TAQMAN® (real-time PCR monitoring of amplification) and Lightcycler quantification of mRNA

Total RNA is prepared from cells following siNA delivery, for example, using Ambion Rnaqueous 4-PCR purification kit for large scale extractions, or Ambion Rnaqueous-96 purification kit for 96-well assays. For Taqman analysis, dual-labeled probes are synthesized with, for example, the reporter dyes FAM or VIC covalently linked at the 5'-end and the quencher dye TAMARA conjugated to the 3'-end. One-step RT-PCR amplifications are performed on, for example, an ABI PRISM 7700 Sequence detector using 50uL reactions consisting of 10uL total RNA, 100nM forward primer, 100nM reverse primer, 100nM probe, 1X TaqMan PCR reaction buffer (PE-Applied Biosystems), 5.5mM MgCl2, 100uM each dATP, dCTP, dGTP and dTTP, 0.2U RNase Inhibitor (Promega), 0.025U AmpliTaq Gold (PE-Applied Biosystems) and 0.2U M-MLV Reverse Transcriptase (Promega). The thermal cycling conditions can consist of 30 minutes at 48°C, 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Quantitation of target mRNA level is determined relative to standards generated from serially diluted total cellular RNA (300, 100, 30, 10 ng/rxn) and normalizing to, for example, 36B4 mRNA in either parallel or same tube TaqMan reactions. For HCV Replicon mRNA quantitation, PCR primers and probe specific for the neomycin gene were used:

neo-forward primer, 5'-CCGGCTACCTGCCCATTC-3'; (SEQ ID NO: 2032)
neo-reverse primer, 5'-CCAGATCATCCTGATCGACAAG-3'; (SEQ ID NO: 2033)
neo-probe, 5'FAM-ACATCGCATCGAGCGAGCACGTAC-TAMARA3'; (SEQ ID NO: 2034)
For normalization, 36B4 PCR primers and probe were used:
36B4-forward primer, 5'-TCTATCATCAACGGGTACAAACGA-3'; (SEQ ID NO: 2035)
36B4 reverse primer, 5'-CTTTTCAGCAAGTGGGAAGGTG-3'; (SEQ ID NO: 2036)
36B4 probe, 5'VIC-CCTGGCCTTGTCTGTGGAGACGGATTA-TAMARA3'; (SEQ ID NO: 2037)

Western blotting

Nuclear extracts can be prepared using a standard micro preparation technique (see for example Andrews and Faller, 1991, *Nucleic Acids Research,* 19, 2499). Protein extracts from supernatants are prepared, for example using TCA precipitation. An equal volume of 20% TCA is added to the cell supernatant, incubated on ice for 1 hour and pelleted by centrifugation for 5 minutes. Pellets are washed in acetone, dried and resuspended in water. Cellular protein extracts are run on a 10% Bis-Tris NuPage (nuclear extracts) or 4-12% Tris-Glycine (supernatant extracts) polyacrylamide gel and transferred onto nitro-cellulose membranes. Non-specific binding can be blocked by incubation, for example, with 5% non-fat milk for 1 hour followed by primary antibody for 16 hour at 4°C. Following washes, the secondary antibody is applied, for example (1:10,000 dilution) for 1 hour at room temperature and the signal detected with SuperSignal reagent (Pierce).

Example 8: Models useful to evaluate the down-regulation of HCV gene expression

Cell Culture

Although there have been reports of replication of HCV in cell culture (see below), these systems are difficult to reproduce and have proven unreliable. Therefore, as was the case for development of other anti-HCV therapeutics, such as interferon and ribavirin, after demonstration of safety in animal studies applicant can proceed directly into a clinical feasibility study.

Several recent reports have documented *in vitro* growth of HCV in human cell lines (Mizutani *et al.,* Biochem Biophys Res Commun 1996 227(3):822-826; Tagawa *et al.,* Journal of Gasteroenterology and Hepatology 1995 10(5):523-527; Cribier *et al., Journal of General Virology* 76(10):2485-2491; Seipp *et al., Journal of General Virology* 1997 78(10)2467-2478; Iacovacci *et al.,* Research Virology 1997 148(2):147-151; Iocavacci *et al.,* Hepatology 1997 26(5) 1328-1337; Ito *et al.,* Journal of General Virology 1996 77(5):1043-1054; Nakajima *et al., Journal of Virology* 1996 70(5):3325-3329; Mizutani *et al., Journal of Virology* 1996 70(10):7219-7223; Valli *et al., Res Virol* 1995 146(4): 285-288; Kato *et al., Biochem Biophys Res Comm* 1995 206(3):863-869). Replication of HCV has been reported in both T and B cell lines, as well as cell lines derived from human hepatocytes. Detection of low level replication was documented

using either RT-PCR based assays or the b-DNA assay. It is important to note that the most recent publications regarding HCV cell cultures document replication for up to 6-months. However, the level of HCV replication observed in these cell lines has not been robust enough for screening of antiviral compounds.

In addition to cell lines that can be infected with HCV, several groups have reported the successful transformation of cell lines with cDNA clones of full-length or partial HCV genomes (Harada *et al.,* Journal of General Virology, 1995, 76(5)1215-1221; Haramatsu *et al.,* Journal of Viral Hepatitis 1997 4S(1):61-67; Dash *et al.,* American Journal of Pathology 1997 151(2):363-373; Mizuno *et al.,* Gasteroenterology 1995 109(6):1933-40; Yoo *et al.,* Journal Of Virology 1995 69(1):32-38).

The recent development of subgenomic HCV RNA replicons capable of successfully replicating in the human hepatoma cell line, Huh7, represents a significant advance toward a dependable cell culture model. These replicons contain the neomycin gene upstream of the HCV nonstructural genes allowing for the selection of replicative RNAs in Huh7 cells. Initially, RNA replication was detected at a low frequency (Lohmann *et al.* Science 1999 285: 110-113) but the identification of replicons with cell-adaptive mutations in the NS5A region has improved the efficiency of replication 10,000-fold (Blight *et al.* Science 2000 290:1972-1975). Steps in the HCV life cycle, such as translation, protein processing, and RNA replication are recapitulated in the subgenomic replicon systems, but early events (viral attachment and uncoating) and viral assembly is absent. Inclusion of the structural genes of HCV within the replicons results in the production of HCV core and envelope proteins, but virus assembly does not occur (Pietschmann *et al.* Journal of Virology 2002 76: 4008-4021). Such replicon systems have been used to study siRNA mediated inhibition of HCV RNA, see for example, Randall *et al.,* 2003, *PNAS USA*, 100, 235-240.

In several cell culture systems, cationic lipids have been shown to enhance the bioavailability of oligonucleotides to cells in culture (Bennet, *et al.,* 1992, *Mol. Pharmacology*, 41, 1023-1033). In one embodiment, siNA molecules of the invention are complexed with cationic lipids for cell culture experiments. siNA and cationic lipid mixtures are prepared in serum-free DMEM immediately prior to addition to the cells. DMEM plus additives are warmed to room temperature (about 20-25°C) and cationic lipid is added to the final desired concentration and the solution is vortexed briefly.

siNA molecules are added to the final desired concentration and the solution is again vortexed briefly and incubated for 10 minutes at room temperature. In dose response experiments, the RNA/lipid complex is serially diluted into DMEM following the 10 minute incubation.

Animal Models

Evaluating the efficacy of anti-HCV agents in animal models is an important prerequisite to human clinical trials. The best characterized animal system for HCV infection is the chimpanzee. Moreover, the chronic hepatitis that results from HCV infection in chimpanzees and humans is very similar. Although clinically relevant, the chimpanzee model suffers from several practical impediments that make use of this model difficult. These include high cost, long incubation requirements and lack of sufficient quantities of animals. Due to these factors, a number of groups have attempted to develop rodent models of chronic hepatitis C infection. While direct infection has not been possible, several groups have reported on the stable transfection of either portions or entire HCV genomes into rodents (Yamamoto *et al.*, Hepatology 1995 22(3): 847-855; Galun *et al.*, Journal of Infectious Disease 1995 172(1):25-30; Koike *et al.*, Journal of general Virology 1995 76(12)3031-3038; Pasquinelli *et al.*, Hepatology 1997 25(3): 719-727; Hayashi *et al.*, Princess Takamatsu Symp 1995 25:1430149; Mariya et al., Journal of General Virology 1997 78(7) 1527-1531; Takehara *et al.*, Hepatology 1995 21(3):746-751; Kawamura *et al.*, Hepatology 1997 25(4): 1014-1021). In addition, transplantation of HCV infected human liver into immunocompromised mice results in prolonged detection of HCV RNA in the animal's blood.

A method for expressing hepatitis C virus in an *in vivo* animal model has been developed (Vierling, International PCT Publication No. WO 99/16307). Viable, HCV infected human hepatocytes are transplanted into a liver parenchyma of a scid/scid mouse host. The scid/scid mouse host is then maintained in a viable state, whereby viable, morphologically intact human hepatocytes persist in the donor tissue and hepatitis C virus is replicated in the persisting human hepatocytes. This model provides an effective means for the study of HCV inhibition by enzymatic nucleic acids *in vivo*.

As such, these models can be used in evaluating the efficacy of siNA molecules of the invention in inhibiting HCV expression. These models and others can similarly be

used to evaluate the safety and efficacy of siNA molecules of the invention in a pre-clinical setting.

Example 9: RNAi mediated inhibition of HCV expression

siNA constructs (**Table III**) are tested for efficacy in reducing HCV RNA expression in, for example, Huh7 cells. Cells are plated approximately 24 hours before transfection in 96-well plates at 5,000-7,500 cells/well, 100 µl/well, such that at the time of transfection cells are 70-90% confluent. For transfection, annealed siNAs are mixed with the transfection reagent (Lipofectamine 2000, Invitrogen) in a volume of 50 µl/well and incubated for 20 minutes at room temperature. The siNA transfection mixtures are added to cells to give a final siNA concentration of 25 nM in a volume of 150 µl. Each siNA transfection mixture is added to 3 wells for triplicate siNA treatments. Cells are incubated at 37° for 24 hours in the continued presence of the siNA transfection mixture. At 24 hours, RNA is prepared from each well of treated cells. The supernatants with the transfection mixtures are first removed and discarded, then the cells are lysed and RNA prepared from each well. Target gene expression following treatment is evaluated by RT-PCR for the target gene and for a control gene (36B4, an RNA polymerase subunit) for normalization. The triplicate data is averaged and the standard deviations determined for each treatment. Normalized data are graphed and the percent reduction of target mRNA by active siNAs in comparison to their respective inverted control siNAs is determined.

Example 10: siNA Inhibition of a chimeric HCV/Poliovirus in HeLa Cells

Inhibition of a chimeric HCV/Poliovirus was investigated using 21 nucleotide siNA duplexes in HeLa cells. Seven siNA were designed that target three regions in the highly conserved 5' untranslated region (UTR) of HCV RNA. The siNAs were screened in two cell culture systems dependent upon the 5'-UTR of HCV; one requires translation of an HCV/luciferase gene, while the other involves replication of a chimeric HCV/poliovirus (PV) (see Blatt *et al.*, USSN 09/740,332, filed December 18, 2000, incorporated by reference herein). Transfection for the HCV/PV system was performed in HeLa cells (grown in DMEM supplemented with sodium pyruvate and 100mM HEPES with 5% FBS) using either cationic lipid NC168 or LFA2K, with an siNA concentration of 10nM or 25nM. HeLa cells were innoculated with HCV/PV virus at an moi=.01 pfu/cell for 30 minutes in serum-free media. The innoculum was removed and

80µL media was added, with 20µL of transfection complex added to each well. The cells and supernatants were frozen at 20-24 hours post transfection. Each plate underwent 3 freeze-thaw cycles and the supernatant was collected. The supernatant was titered on HeLa cells for 3 days, then stained and counted. The results shown in **Figures 24-27** are reported as pfu/ml x 10^5.

Two siNAs (29579/29586 and 29578/29585, see **Table III**) targeting the same region (shifted by one nucleotide) are active in both systems (see **Figure 22**). For example, a >85% reduction in HCVPV replication was observed in siNA-treated cells compared to an inverse siNA control 29593/29600 (**Figure 22**) with an IC50 = ~2.5 nM (**Figure 23**). To develop nuclease-resistant siNA for in vivo applications, siNAs can be modified to contain stabilizing chemical modifications. Such modifications include phosphorothioate linkages (P=S), 2'–*O*–methyl nucleotides, 2'–fluoro (F) nucleotides, 2'-deoxy nucleotides, universal base nucleotides, 5' and/or 3' end modifications and a variety of other nucleotide and non-nucleotide modifications, such as those described herein, in one or both siNA strands. Using this systematic approach, active siNA molecules have been identified that are substantially more resistant to nucleases. Several of these constructs were tested in the HCV/poliovirus chimera system, demonstrating significant reduction in viral replication (see **Figures 24-27**). siNA constructs shown in **Figures 24-27** are referred to by Compound numbers that are cross referenced and identified in **Table III**. siNA activity is compared to relevant controls (untreated cells, scrambled/inactive control sequences, or transfection controls). **Figure 24** shows the inhibition of HCV RNA in the HCV/poliovirus chimera system using chemically modified siNA construct 30051/30053, which construct has inverted deoxy abasic nucleotides at the 3' and 5' ends, several phosphorothioate linkages, and 5-nitroindole nucleotides. **Figure 25** shows the inhibition of HCV RNA in the HCV/poliovirus chimera system using chemically modified siNA construct 30055/30057, which construct has inverted deoxy abasic nucleotides at the 3' and 5' ends, several phosphorothioate linkages, and 5-nitroindole nucleotides. **Figures 26 and 27** show the inhibition of HCV RNA in the HCV/poliovirus chimera system using unmodified siNA construct (29586/29579) and chemically modified siNA constructs 30417/30419, 30417/30420, 30418/30419, and combinations thereof at 10 nM and 25 nM siNA, respectively. As shown in **Figures 24-27**, siNA constructs of the invention provide potent inhibition of HCV RNA in the HCV/poliovirus chimera system. As such, siNA

constructs, inlcuding chemically modified, nuclease resistant siNA molecules, represent an important class of therapeutic agents for treating chronic HCV infection.

Example 11: siNA Inhibition of HCV RNA expression in a HCV replicon system

A HCV replicon system was used to test the efficacy of siNAs targeting HCV RNA. The reagents are tested in cell culture using Huh7 cells (see for example Randall *et al.,* 2003, *PNAS USA*, 100, 235-240) to determine the extent of RNA and protein inhibition. siNA were selected against the HCV target as described herein. RNA inhibition was measured after delivery of these reagents by a suitable transfection agent to Huh7 cells. Relative amounts of target RNA are measured versus actin using real-time PCR monitoring of amplification (*eg.*, ABI 7700 Taqman®). A comparison is made to a mixture of oligonucleotide sequences designed to target unrelated targets or to a randomized siNA control with the same overall length and chemistry, but with randomly substituted nucleotides at each position. Primary and secondary lead reagents were chosen for the target and optimization performed. After an optimal transfection agent concentration is chosen, a RNA time-course of inhibition is performed with the lead siNA molecule. In addition, a cell-plating format can be used to determine RNA inhibition. A non-limiting example of a multiple target screen to assay siNA mediated inhibition of HCV RNA is shown in **Figure 28**. siNA reagents **(Table III)** were transfected at 25 nM into Huh7 cells and HCV RNA quantitated compared to untreated cells ("cells" column in the figure),cells transfected with lipofectamine ("LFA2K" column in the figure) and matched chemistry inverted controls ("Inv"). As shown in the Figure, several siNA constructs show significant inhibition of HCV RNA expression in the Huh7 replicon system. Chemically modified siNA constructs were then screened as described above, with a non-limiting example of a Stab 7/8 (see **Table IV**) chemisty siNA construct screen shown in **Figure 30**. A follow up dose response study using chemically modified siNA constructs (Stab 4/5, see **Table IV**) at concentrations of 5nM, 10nM, 25 nM and 100 nM compared to matched chemistry inverted controls is shown in **Figure 29**, whereas a dose response study for Stab 7/8 constructs at concentrations of 5nM, 10nM, 25 nM, 50 nM and 100 nM compared to matched chemistry inverted controls is shown in **Figure 31**.

Example 12: Effect of Interferon/siNA Combination Treatment on Replication of HCV Subgenomic Replicon in Huh7 Cells

To investigate combination use of RNAi and interferon in the inhibition of HCV replication, siNA and interferon combination treatments were assayed in the HCV Subgenomic Replicon in Huh7 cells. Huh7 cells containing the HCV subgenomic replicon Clone A were plated in 96-well plates at a density of 9,600 cells per well and incubated overnight at 37°C. The cells were then treated with interferon alone, siNAs or inverted sequence controls alone, or with interferon in combination with siNAs or inverted controls. A sub-optimal dose of interferon was used in order to observe possible potentiation of the interferon anti-viral activity in the presence of the HCV-targeted siNA. The cells were transfected with HCV targeted siNAs (31703/31707) or inverted sequence controls (31711/31715) at 5, 10, 25, 50, or 100 nM using 0.35 ul/well of Lipofectamine 2000 in media alone, or media to which was added 1.7 Units/ml of Infergen (Amgen). The cells were then incubated at 37°C for 48 or 72 hours, at which time total RNA was isolated using an Invitek 96-well RNA isolation kit. To quantitate the levels of RNA from the HCV replicon, real-time RT-PCR was performed using probes and primers to the neomycin resistance region of the replicon. Results are shown in **Figure 32**. Levels of the replicon RNA were normalized to the levels of cellular GAPDH mRNA. These data demonstrate potentiation of the effect of combination siNA/interferon treatment compared to interferon alone.

Example 13: Multifunctional siNA Inhibition of HCV RNA expression in a HCV replicon system

To investigate the use of multifunctional siNA constructs that target different sites within HCV RNA via RNAi, multifunctional siNA treatments were assayed in the HCV Subgenomic Replicon in Huh7 cells. The multifunctional siNA constructs that target either sites 304 and 327 or sites 282 and 304 of HCV RNA were compared to pools of siNA molecules that individually target site 304 and site 327 or site 282 and site 304 of HCV RNA. Huh7 cells containing the HCV subgenomic replicon Clone A were plated in 96-well plates at a density of 9,600 cells per well and incubated overnight at 37°C. The cells were then treated with the individual siNAs, pooled siNAs, multifunctional siNAs, irrelevant multifunctional siNA controls, and a transfection control (LFA only). The cells were then incubated at 37°C for 48 or 72 hours, at which time total RNA was

isolated using an Invitek 96-well RNA isolation kit. To quantitate the levels of RNA from the HCV replicon, real-time RT-PCR was performed using probes and primers to the neomycin resistance region of the replicon. Dose response results for unmodified multifunctional siNA constructs assayed at 0.1, 1.0 and 10 nM are shown in **Figure 33**, whereas results for modified multifunctional siNA constructs are shown in **Figures 34-41**. Levels of the replicon RNA were normalized to the levels of cellular GAPDH mRNA. These data demonstrate that the multifunctional siNA constructs are equally effective in inhibition of HCV RNA expression by targeting multiple sites as are pools of individual siNA constructs that target each site.

Example 14: Multifunctional siNA design

Once target sites have been identified for multifunctional siNA constructs, each strand of the siNA is designed with a complementary region of length, for example, between about 18 and about 28 nucleotides, that is complementary to a different target nucleic acid sequence. Each complementary region is designed with an adjacent flanking region of about 4 to about 22 nucleotides that is not complementary to the target sequence, but which comprises complementarity to the complementary region of the other sequence (see for example **Figure 16**). Hairpin constructs can likewise be designed (see for example **Figure 17**). Identification of complementary, palindrome or repeat sequences that are shared between the different target nucleic acid sequences can be used to shorten the overall length of the multifunctional siNA constructs (see for example **Figures 18 and 19**).

In a non-limiting example, a multifunctional siNA is designed to target two separate nucleic acid sequences. The goal is to combine two different siNAs together in one siNA that is active against two different targets. The siNAs are joined in a way that the 5' of each strand starts with the "antisense" sequence of one of two siRNAs as shown in italics below.

3' TTAGAAACCAGACGUAAGUGU GGUACGACCUGACGACCGU 5' SEQ ID NO: 2038

5' *UCUUUGGUCUGCAUUCACA*C CAUGCUGGACUGCUGGCATT3' SEQ ID NO: 2039

RISC is expected to incorporate either of the two strands from the 5' end. This would lead to two types of active RISC populations carrying either strand. The 5' 19 nt of each strand will act as guide sequence for degradation of separate target sequences.

In another example, the size of multifunctional siNA molecules is reduced by either finding overlaps or truncating the individual siNA length. The exemplary excercise described below indicates that for any given first target sequence, a shared complementary sequence in a second target sequence is likely to be found.

The number of spontaneous matches of short polynucleotide sequences (e.g., less than 14 nucleotides) that are expected to occur between two longer sequences generated independent of one another was investigated. A simulation using the uniform random generator SAS V8.1 utilized a 4,000 character string that was generated as a random repeating occurrence of the letters {ACGU}. This sequence was then broken into the nearly 4000 overlapping sets formed by taking S1 as the characters from positions (1,2...n), S2 from positions (2,3..., n+1) completely through the sequence to the last set, S 4000-n+1 from position (4000-n+1,...,4000). This process was then repeated for a second 4000 character string. Occurrence of same sets (of size n) were then checked for sequence identity between the two strings by a sorting and match-merging routine. This procedure was repeated for sets of 9-11 characters. Results were an average of 55 matching sequences of length n= 9 characters (range 39 to 72); 13 common sets (range 6 to 18) for size n=10, and 4 matches on average (range 0 to 6) for sets of 11 characters. The choice of 4000 for the original string length is approximately the length of the coding region of both target nucleic acid molecules. This simple simulation suggests that any two long coding regions formed independent of one-another will share common short sequences that can be used to shorten the length of multifunctional siNA constructs. In this example, common sequences of size 9 occurred by chance alone in > 1% frequency.

In another example, the length of a multifunctional siNA construct is reduced by determining whether fewer base pairs of sequence homology to each target sequence can be tolerated for effective RNAi activity. If so, the overall length of multifunctional siNA can be reduced as shown below. In the following hypothetical example, 4 nucleotides (bold) are reduced from each 19 nucleotide siNA '1' and siNA '2' constructs. The resulting multifunctional siNA is 30 base pairs long.

Additional Multifuctional siNA Designs

Three categories of additional multifunctional siNA designs are presented that allow a single siNA molecule to silence multiple targets. The first method utilizes linkers to join siNAs (or multiunctional siNAs) in a direct manner. This can allow the most potent siNAs to be joined without creating a long, continuous stretch of RNA that has potential to trigger an interferon response. The second method is a dendrimeric extension of the overlapping or the linked multifunctional design; or alternatively the organization of siNA in a supramolecular format. The third method uses helix lengths greater than 30 base pairs. Processing of these siNAs by Dicer will reveal new, active 5' antisense ends. Therefore, the long siNAs can target the sites defined by the original 5' ends and those defined by the new ends that are created by Dicer processing. When used in combination with traditional multifunctional siNAs (where the sense and antisense strands each define a target) the approach can be used for example to target 4 or more sites.

I. Tethered Bifunctional siNAs

The basic idea is a novel approach to the design of multifunctional siNAs in which two antisense siNA strands are annealed to a single sense strand. The sense strand oligonucleotide contains a linker (e.g., non-nulcoetide linker as described herein) and two segments that anneal to the antisense siNA strands (see **Figure 42**). The linkers can also optionally comprise nucleotide-based linkers. Several potential advantages and variations to this approach include, but are not limited to:

1. The two antisense siNAs are independent. Therefore, the choice of target sites is not constrained by a requirement for sequence conservation between two sites. Any two highly active siNAs can be combined to form a multifunctional siNA.

2. When used in combination with target sites having homology, siNAs that target a sequence present in two genes (e.g., different HCV strains), the design can be used to target more than two sites. A single multifunctional siNA can be for example, used to target RNA of two different HCV RNAs (using one antisense strand of the multifunctional siNA targeting of conserved sequence between to the two RNAs) and a host RNA (using the second antisense strand of the multifunctional siNA targeting host RNA (e.g., La antigen or FAS) This approach allows targeting of

more than one HCV strain and one or more host RNAs using a single multifunctional siNA.

3. Multifunctional siNAs that use both the sense and antisense strands to target a gene can also be incorporated into a tethered multifuctional design. This leaves open the possibility of targeting 6 4 or more sites with a single complex.

4. It can be possible to anneal more than two antisense strand siNAs to a single tethered sense strand.

5. The design avoids long continuous stretches of dsRNA. Therefore, it is less likely to initiate an interferon response.

6. The linker (or modifications attached to it, such as conjugates described herein) can improve the pharmacokinetic properties of the complex or improve its incorporation into liposomes. Modifications introduced to the linker should not impact siNA activity to the same extent that they would if directly attached to the siNA (see for example **Figures 48 and 49**).

7. The sense strand can extend beyond the annealed antisense strands to provide additional sites for the attachment of conjugates.

8. The polarity of the complex can be switched such that both of the antisense 3' ends are adjacent to the linker and the 5' ends are distal to the linker or combination thereof.

Dendrimer and supramolecular siNAs

In the dendrimer siNA approach, the synthesis of siNA is initiated by first synthesizing the dendrimer template followed by attaching various functional siNAs. Various constructs are depicted in **Figure 43**. The number of functional siNAs that can be attached is only limited by the dimensions of the dendrimer used.

Supramolecular approach to multifunctional siNA

The supramolecular format simplifies the challenges of dendrimer synthesis. In this format, the siNA strands are synthesized by standard RNA chemistry, followed by

annealing of various complementary strands. The individual strand synthesis contains an antisense sense sequence of one siNA at the 5'-end followed by a nucleic acid or synthetic linker, such as hexaethyleneglyol, which in turn is followed by sense strand of another siNA in 5' to 3' direction. Thus, the synthesis of siNA strands can be carried out in a standard 3' to 5' direction. Representative examples of trifunctional and tetrafunctional siNAs are depicted in **Figure 44**. Based on a similar principle, higher functionality siNA constucts can be designed as long as efficient annealing of various strands is achieved.

Dicer enabled multifunctional siNA

Using bioinformatic analysis of multiple targets, stretches of identical sequences shared between differeing target sequences can be identified ranging from about two to about fourteen nucleotides in length. These identical regions can be designed into extended siNA helixes (e.g., >30 base pairs) such that the processing by Dicer reveals a secondary functional 5'-antisense site (see for example **Figure 45**). For example, when the first 17 nucleotides of a siNA antisense strand (e.g., 21 nucleotide strands in a duplex with 3'-TT overhangs) are complementary to a target RNA, robust silencing was observed at 25 nM. 80% silencing was observed with only 16 nucleotide complementarity in the same format (see **Figure 47**).

Incorporation of this property into the designs of siNAs of about 30 to 40 or more base pairs results in additional multifunctional siNA constructs. The example in **Figure 45** illustrates how a 30 base-pair duplex can target three distinct sequences after processing by Dicer-RNaseIII; these sequences can be on the same mRNA or separate RNAs, such as viral and host factor messages, or multiple points along a given pathway (e.g., inflammatory cascades). Furthermore, a 40 base-pair duplex can combine a bifunctional design in tandem, to provide a single duplex targeting four target sequences. An even more extensive approach can include use of homologous sequences (e.g. VEGFR-1/VEGFR-2) to enable five or six targets silenced for one multifunctional duplex. The example in **Figure 45** demonstrates how this can be achieved. A 30 base pair duplex is cleaved by Dicer into 22 and 8 base pair products from either end (8 b.p. fragments not shown). For ease of presentation the overhangs generated by dicer are not shown – but can be compensated for. Three targeting sequences are shown. The required sequence identity overlapped is indicated by grey boxes. The N's of the parent

30 b.p. siNA are suggested sites of 2'-OH positions to enable Dicer cleavage if this is tested in stabilized chemistries. Note that processing of a 30mer duplex by Dicer RNase III does not give a precise 22+8 cleavage, but rather produces a series of closely related products (with 22+8 being the primary site). Therefore, processing by Dicer will yield a series of active siNAs. Another non-limiting example is shown in **Figure 46**. A 40 base pair duplex is cleaved by Dicer into 20 base pair products from either end. For ease of presentation the overhangs generated by dicer are not shown – but can be compensated for. Four targeting sequences are shown in four colors, blue, light-blue and red and orange. The required sequence identity overlapped is indicated by grey boxes. This design format can be extended to larger RNAs. If chemically stabilized siNAs are bound by Dicer, then strategically located ribonucleotide linkages can enable designer cleavage products that permit our more extensive repertoire of multiifunctional designs. For example cleavage products not limited to the Dicer standard of approximately 22-nucleotides can allow multifunctional siNA constructs with a target sequence identity overlap ranging from, for example, about 3 to about 15 nucleotides.

Another important aspect of this approach is its ability to restrict escape mutants. Processing to reveal an internal target site can ensure that escape mutations complementary to the eight nucleotides at the antisense 5' end will not reduce siNA effectiveness. If about 17 nucleotidest of complementarity are required for RISC-mediated target cleavage, this will restrict, for example 8/17 or 47% of potential escape mutants.

Example 15: Indications

The present body of knowledge in HCV research indicates the need for methods to assay HCV activity and for compounds that can regulate HCV expression for research, diagnostic, and therapeutic use. As described herein, the nucleic acid molecules of the present invention can be used in assays to diagnose disease state related of HCV levels. In addition, the nucleic acid molecules can be used to treat disease state related to HCV levels.

Particular degenerative and disease states that can be associated with HCV expression modulation include, but are not limited to, HCV infection, liver failure, hepatocellular carcinoma, cirrhosis, and/or other disease states associated with HCV infection.

Example 16: Interferons

Interferons represent a non-limiting example of a class of compounds that can be used in conjuction with the siNA molecules of the invention for treating the diseases and/or conditions described herein. Type I interferons (IFN) are a class of natural cytokines that includes a family of greater than 25 IFN-α (Pesta, 1986, *Methods Enzymol.* 119, 3-14) as well as IFN-β, and IFN-ω. Although evolutionarily derived from the same gene (Diaz *et al.*, 1994, *Genomics* 22, 540-552), there are many differences in the primary sequence of these molecules, implying an evolutionary divergence in biologic activity. All type I IFN share a common pattern of biologic effects that begin with binding of the IFN to the cell surface receptor (Pfeffer & Strulovici, 1992, Transmembrane secondary messengers for IFN-α/β. In: *Interferon. Principles and Medical Applications.*, S. Baron, D.H. Coopenhaver, F. Dianzani, W.R. Fleischmann Jr., T.K. Hughes Jr., G.R. Kimpel, D.W. Niesel, G.J. Stanton, and S.K. Tyring, eds. 151-160). Binding is followed by activation of tyrosine kinases, including the Janus tyrosine kinases and the STAT proteins, which leads to the production of several IFN-stimulated gene products (Johnson *et al.*, 1994, *Sci. Am.* 270, 68-75). The IFN-stimulated gene products are responsible for the pleotropic biologic effects of type I IFN, including antiviral, antiproliferative, and immunomodulatory effects, cytokine induction, and HLA class I and class II regulation (Pestka *et al.*, 1987, *Annu. Rev. Biochem* 56, 727). Examples of IFN-stimulated gene products include 2-5-oligoadenylate synthetase (2-5 OAS), β_2-microglobulin, neopterin, p68 kinases, and the Mx protein (Chebath & Revel, 1992, The 2-5 A system: 2-5 A synthetase, isospecies and functions. In: *Interferon. Principles and Medical Applications,* S. Baron, D.H. Coopenhaver, F. Dianzani, W.R. Jr. Fleischmann, T.K. Jr Hughes, G.R. Kimpel, D.W. Niesel, G.J. Stanton, and S.K. Tyring, eds., pp. 225-236; Samuel, 1992, The RNA-dependent P1/eIF-2α protein kinase. In: *Interferon. Principles and Medical Applications.* S. Baron, D.H. Coopenhaver, F. Dianzani, W.R. Fleischmann Jr., T.K. Hughes Jr., G.R. Kimpel, D.W. Niesel, G.H. Stanton, and S.K. Tyring, eds. 237-250; Horisberger, 1992, MX protein: function and Mechanism of Action. In: *Interferon. Principles and Medical Applications.* S. Baron, D.H. Coopenhaver, F. Dianzani, W.R. Fleischmann Jr., T.K. Hughes Jr., G.R. Kimpel, D.W. Niesel, G.H. Stanton, and S.K. Tyring, eds. 215-224). Although all type I IFN have similar biologic effects, not all the activities are shared by each type I IFN, and in many cases, the extent of activity varies quite substantially for each IFN subtype (Fish *et*

al, 1989, *J. Interferon Res.* 9, 97-114; Ozes *et al.*, 1992, *J. Interferon Res.* 12, 55-59). More specifically, investigations into the properties of different subtypes of IFN-α and molecular hybrids of IFN-α have shown differences in pharmacologic properties (Rubinstein, 1987, *J. Interferon Res.* 7, 545-551). These pharmacologic differences can arise from as few as three amino acid residue changes (Lee *et al.*, 1982, *Cancer Res.* 42, 1312-1316).

Eighty-five to 166 amino acids are conserved in the known IFN-α subtypes. Excluding the IFN-α pseudogenes, there are approximately 25 known distinct IFN-α subtypes. Pairwise comparisons of these nonallelic subtypes show primary sequence differences ranging from 2% to 23%. In addition to the naturally occurring IFNs, a non-natural recombinant type I interferon known as consensus interferon (CIFN) has been synthesized as a therapeutic compound (Tong *et al.*, 1997, *Hepatology* 26, 747-754).

Interferon is currently in use for at least 12 different indications, including infectious and autoimmune diseases and cancer (Borden, 1992, *N. Engl. J. Med.* 326, 1491-1492). For autoimmune diseases, IFN has been utilized for treatment of rheumatoid arthritis, multiple sclerosis, and Crohn's disease. For treatment of cancer, IFN has been used alone or in combination with a number of different compounds. Specific types of cancers for which IFN has been used include squamous cell carcinomas, melanomas, hypernephromas, hemangiomas, hairy cell leukemia, and Kaposi's sarcoma. In the treatment of infectious diseases, IFNs increase the phagocytic activity of macrophages and cytotoxicity of lymphocytes and inhibits the propagation of cellular pathogens. Specific indications for which IFN has been used as treatment include hepatitis B, human papillomavirus types 6 and 11 (i.e. genital warts) (Leventhal *et al.*, 1991, *N Engl J Med* 325, 613-617), chronic granulomatous disease, and hepatitis C virus.

Numerous well controlled clinical trials using IFN-alpha in the treatment of chronic HCV infection have demonstrated that treatment three times a week results in lowering of serum ALT values in approximately 50% (range 40% to 70%) of patients by the end of 6 months of therapy (Davis *et al.*, 1989, N. Engl. J. Med. 321, 1501-1506; Marcellin et al., 1991, *Hepatology* 13, 393-397; Tong *et al.*, 1997, Hepatology 26, 747-754; Tong et al., *Hepatology* 26, 1640-1645). However, following cessation of interferon treatment, approximately 50% of the responding patients relapsed, resulting in a

"durable" response rate as assessed by normalization of serum ALT concentrations of approximately 20 to 25%. In addition, studies that have examined six months of type 1 interferon therapy using changes in HCV RNA values as a clinical endpoint have demonstrated that up to 35% of patients will have a loss of HCV RNA by the end of therapy (Tong *et al.*, 1997, supra). However, as with the ALT endpoint, about 50% of the patients relapse six months following cessation of therapy resulting in a durable virologic response of only 12% (23). Studies that have examined 48 weeks of therapy have demonstrated that the sustained virological response is up to 25%.

Pegylated interferons, i.e., interferons conjugated with polyethylene glycol (PEG), have demonstrated improved characteristics over interferon. Advantages incurred by PEG conjugation can include an improved pharmacokinetic profile compared to interferons lacking PEG, thus imparting more convenient dosing regimes, improved tolerance, and improved antiviral efficacy. Such improvements have been demonstrated in clinical studies of both polyethylene glycol interferon alfa-2a (PEGASYS, Roche) and polyethylene glycol interferon alfa-2b (VIRAFERON PEG, PEG-INTRON, Enzon/Schering Plough).

siNA molecules in combination with interferons and polyethylene glycol interferons have the potential to improve the effectiveness of treatment of HCV or any of the other indications discussed above. siNA molecules targeting RNAs associated with HCV infection can be used individually or in combination with other therapies such as interferons and polyethylene glycol interferons and to achieve enhanced efficacy.

Example 17: Diagnostic uses

The siNA molecules of the invention can be used in a variety of diagnostic applications, such as in the identification of molecular targets (e.g., RNA) in a variety of applications, for example, in clinical, industrial, environmental, agricultural and/or research settings. Such diagnostic use of siNA molecules involves utilizing reconstituted RNAi systems, for example, using cellular lysates or partially purified cellular lysates. siNA molecules of this invention can be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of endogenous or exogenous, for example viral, RNA in a cell. The close relationship between siNA activity and the structure of the target RNA allows the detection of mutations in any region of the molecule, which alters the base-pairing and three-dimensional structure of

the target RNA. By using multiple siNA molecules described in this invention, one can map nucleotide changes, which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with siNA molecules can be used to inhibit gene expression and define the role of specified gene products in the progression of disease or infection. In this manner, other genetic targets can be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combination therapies (e.g., multiple siNA molecules targeted to different genes, siNA molecules coupled with known small molecule inhibitors, or intermittent treatment with combinations siNA molecules and/or other chemical or biological molecules). Other *in vitro* uses of siNA molecules of this invention are well known in the art, and include detection of the presence of mRNAs associated with a disease, infection, or related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a siNA using standard methodologies, for example, fluorescence resonance emission transfer (FRET).

In a specific example, siNA molecules that cleave only wild-type or mutant forms of the target RNA are used for the assay. The first siNA molecules (*i.e.*, those that cleave only wild-type forms of target RNA) are used to identify wild-type RNA present in the sample and the second siNA molecules (*i.e.*, those that cleave only mutant forms of target RNA) are used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA are cleaved by both siNA molecules to demonstrate the relative siNA efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus, each analysis requires two siNA molecules, two substrates and one unknown sample, which is combined into six reactions. The presence of cleavage products is determined using an RNase protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (*i.e.*, disease related or infection related) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts,

then a qualitative comparison of RNA levels is adequate and decreases the cost of the initial diagnosis. Higher mutant form to wild-type ratios are correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications can be made to the invention disclosed herein without departing from the scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims. The present invention teaches one skilled in the art to test various combinations and/or substitutions of chemical modifications described herein toward generating nucleic acid constructs with improved activity for mediating RNAi activity. Such improved activity can comprise improved stability, improved bioavailability, and/or improved activation of cellular responses mediating RNAi. Therefore, the specific embodiments described herein are not limiting and one skilled in the art can readily appreciate that specific combinations of the modifications described herein can be tested without undue experimentation toward identifying siNA molecules with improved RNAi activity.

The invention illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use

of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

Table I: HCV Accession Numbers

Seq Name	Acc#	LOCUS
gi|329763|gb|M84754.1|HPCGENANTI	M84754.1	HPCGENANTI
gi|567059|gb|U16362.1|HCU16362	U16362.1	HCU16362
gi|5918956|gb|AF165059.1|AF165059	AF165059.1	AF165059
gi|385583|gb|S62220.1|S62220	S62220.1	S62220
gi|6010587|gb|AF177040.1|AF177040	AF177040.1	AF177040
gi|5748510|emb|AJ238800.1|HCJ238800	AJ238800.1	HCJ238800
gi|7650221|gb|AF207752.1|AF207752	AF207752.1	AF207752
gi|11559454|dbj|AB049094.1|AB049094	AB049094.1	AB049094
gi|3550760|dbj|D84263.1|D84263	D84263.1	D84263
gi|221610|dbj|D90208.1|HPCJCG	D90208.1	HPCJCG
gi|558520|dbj|D28917.1|HPCK3A	D28917.1	HPCK3A
gi|2176577|dbj|E08461.1|E08461	E08461.1	E08461
gi|6707285|gb|AF169005.1|AF169005	AF169005.1	AF169005
gi|12309923|emb|AX057094.1|AX057094	AX057094.1	AX057094
gi|6010585|gb|AF177039.1|AF177039	AF177039.1	AF177039
gi|7329202|gb|AF238482.1|AF238482	AF238482.1	AF238482
gi|11559464|dbj|AB049099.1|AB049099	AB049099.1	AB049099
gi|5918932|gb|AF165047.1|AF165047	AF165047.1	AF165047
gi|5918946|gb|AF165054.1|AF165054	AF165054.1	AF165054
gi|7650233|gb|AF207758.1|AF207758	AF207758.1	AF207758
gi|19568932|gb|AF483269.1|	AF483269.1	
gi|7650247|gb|AF207765.1|AF207765	AF207765.1	AF207765
gi|12309919|emb|AX057086.1|AX057086	AX057086.1	AX057086
gi|5708597|dbj|E10839.1|E10839	E10839.1	E10839
gi|2327074|gb|AF011753.1|AF011753	AF011753.1	AF011753
gi|12310062|emb|AX057317.1|AX057317	AX057317.1	AX057317
gi|221606|dbj|D10750.1|HPCJ491	D10750.1	HPCJ491
gi|2174448|dbj|E06261.1|E06261	E06261.1	E06261
gi|3098640|gb|AF054251.1|AF054251	AF054251.1	AF054251
gi|18027684|gb|AF313916.1|AF313916	AF313916.1	AF313916
gi|329873|gb|M62321.1|HPCPLYPRE	M62321.1	HPCPLYPRE
gi|464177|dbj|D14853.1|HPCCGS	D14853.1	HPCCGS
gi|15422182|gb|AY051292.1|	AY051292.1	
gi|676877|dbj|D49374.1|HPCFG	D49374.1	HPCFG
gi|1030706|dbj|D50480.1|HPCK1R1	D50480.1	HPCK1R1
gi|7650223|gb|AF207753.1|AF207753	AF207753.1	AF207753
gi|7650237|gb|AF207760.1|AF207760	AF207760.1	AF207760
gi|11559444|dbj|AB049089.1|AB049089	AB049089.1	AB049089
gi|3550762|dbj|D84264.1|D84264	D84264.1	D84264
gi|12831192|gb|AF333324.1|AF333324	AF333324.1	AF333324
gi|13122265|dbj|AB047641.1|AB047641	AB047641.1	AB047641
gi|7329204|gb|AF238483.1|AF238483	AF238483.1	AF238483
gi|11559468|dbj|AB049101.1|AB049101	AB049101.1	AB049101
gi|5918934|gb|AF165048.1|AF165048	AF165048.1	AF165048
gi|5918948|gb|AF165055.1|AF165055	AF165055.1	AF165055
gi|7650235|gb|AF207759.1|AF207759	AF207759.1	AF207759
gi|7650249|gb|AF207766.1|AF207766	AF207766.1	AF207766
gi|9843676|emb|AJ278830.1|HEC278830	AJ278830.1	HEC278830
gi|11559450|dbj|AB049092.1|AB049092	AB049092.1	AB049092
gi|2943783|dbj|D89815.1|D89815	D89815.1	D89815
gi|9626438|ref|NC_001433.1|	NC_001433.1	

gi|12310134|emb|AX057395.1|AX057395	AX057395.1	AX057395
gi|11559460|dbj|AB049097.1|AB049097	AB049097.1	AB049097
gi|12309922|emb|AX057092.1|AX057092	AX057092.1	AX057092
gi|2174644|dbj|E06457.1|E06457	E06457.1	E06457
gi|2176559|dbj|E08443.1|E08443	E08443.1	E08443
gi|5918960|gb|AF165061.1|AF165061	AF165061.1	AF165061
gi|2326454|emb|Y12083.1|HCV12083	Y12083.1	HCV12083
gi|5918938|gb|AF165050.1|AF165050	AF165050.1	AF165050
gi|7650225|gb|AF207754.1|AF207754	AF207754.1	AF207754
gi|7650261|gb|AF207772.1|AF207772	AF207772.1	AF207772
gi|1030704|dbj|D50485.1|HPCK1S2	D50485.1	HPCK1S2
gi|3550758|dbj|D84262.1|D84262	D84262.1	D84262
gi|7650239|gb|AF207761.1|AF207761	AF207761.1	AF207761
gi|3550764|dbj|D84265.1|D84265	D84265.1	D84265
gi|7329206|gb|AF238484.1|AF238484	AF238484.1	AF238484
gi|2176516|dbj|E08399.1|E08399	E08399.1	E08399
gi|5918936|gb|AF165049.1|AF165049	AF165049.1	AF165049
gi|11559446|dbj|AB049090.1|AB049090	AB049090.1	AB049090
gi|5441837|emb|AJ242653.1|SSE242653	AJ242653.1	SSE242653
gi|3098641|gb|AF054252.1|AF054252	AF054252.1	AF054252
gi|4753720|emb|AJ132997.1|HCV132997	AJ132997.1	HCV132997
gi|5420376|emb|AJ238799.1|HCJ238799	AJ238799.1	HCJ238799
gi|11559440|dbj|AB049087.1|AB049087	AB049087.1	AB049087
gi|15529110|gb|AY045702.1|	AY045702.1	
gi|560788|dbj|D30613.1|HPCPP	D30613.1	HPCPP
gi|11225869|emb|AX036253.1|AX036253	AX036253.1	AX036253
gi|11559456|dbj|AB049095.1|AB049095	AB049095.1	AB049095
gi|329770|gb|M58335.1|HPCHUMR	M58335.1	HPCHUMR
gi|6707279|gb|AF169002.1|AF169002	AF169002.1	AF169002
gi|221586|dbj|D10749.1|HPCHCJ1	D10749.1	HPCHCJ1
gi|2171981|dbj|E03766.1|E03766	E03766.1	E03766
gi|6010579|gb|AF177036.1|AF177036	AF177036.1	AF177036
gi|1030703|dbj|D50484.1|HPCK1S3	D50484.1	HPCK1S3
gi|3098650|gb|AF054257.1|AF054257	AF054257.1	AF054257
gi|5821154|dbj|AB016785.1|AB016785	AB016785.1	AB016785
gi|5918962|gb|AF165062.1|AF165062	AF165062.1	AF165062
gi|7650227|gb|AF207755.1|AF207755	AF207755.1	AF207755
gi|7650263|gb|AF207773.1|AF207773	AF207773.1	AF207773
gi|1183030|dbj|D63822.1|HPCJK046E2	D63822.1	HPCJK046E2
gi|13122271|dbj|AB047644.1|AB047644	AB047644.1	AB047644
gi|2443428|gb|U89019.1|HCU89019	U89019.1	HCU89019
gi|2462303|emb|Y13184.1|HCV1480	Y13184.1	HCV1480
gi|7329208|gb|AF238485.1|AF238485	AF238485.1	AF238485
gi|1160327|dbj|D14484.1|HPCJRNA	D14484.1	HPCJRNA
gi|12309921|emb|AX057090.1|AX057090	AX057090.1	AX057090
gi|3098643|gb|AF054253.1|AF054253	AF054253.1	AF054253
gi|21397075|gb|AF511948.1|	AF511948.1	
gi|1030701|dbj|D50482.1|HPCK1R3	D50482.1	HPCK1R3
gi|1030702|dbj|D50483.1|HPCK1S1	D50483.1	HPCK1S1
gi|3098632|gb|AF054247.1|AF054247	AF054247.1	AF054247
gi|59478|emb|X61596.1|HCVJK1G	X61596.1	HCVJK1G
gi|3098652|gb|AF054258.1|AF054258	AF054258.1	AF054258
gi|5918950|gb|AF165056.1|AF165056	AF165056.1	AF165056
gi|7650251|gb|AF207767.1|AF207767	AF207767.1	AF207767
gi|5918964|gb|AF165063.1|AF165063	AF165063.1	AF165063
gi|5918928|gb|AF165045.1|AF165045	AF165045.1	AF165045

gi|5532421|gb|AF139594.1|AF139594	AF139594.1	AF139594
gi|13122267|dbj|AB047642.1|AB047642	AB047642.1	AB047642
gi|5441831|emb|AJ242651.1|SSE242651	AJ242651.1	SSE242651
gi|7650265|gb|AF207774.1|AF207774	AF207774.1	AF207774
gi|7650229|gb|AF207756.1|AF207756	AF207756.1	AF207756
gi|1183032|dbj|D63821.1|HPCJK049E1	D63821.1	HPCJK049E1
gi|2175714|dbj|E07579.1|E07579	E07579.1	E07579
gi|1212741|dbj|D45172.1|HPCHCPO	D45172.1	HPCHCPO
gi|5708511|dbj|E05027.1|E05027	E05027.1	E05027
gi|1483141|dbj|D50409.1|D50409	D50409.1	D50409
gi|13122261|dbj|AB047639.1|AB047639	AB047639.1	AB047639
gi|6521008|dbj|AB031663.1|AB031663	AB031663.1	AB031663
gi|633201|emb|X76918.1|HCVCENS1	X76918.1	HCVCENS1
gi|329737|gb|M67463.1|HPCCGAA	M67463.1	HPCCGAA
gi|11559452|dbj|AB049093.1|AB049093	AB049093.1	AB049093
gi|13619567|emb|AX100563.1|AX100563	AX100563.1	AX100563
gi|221604|dbj|D13558.1|HPCJ483	D13558.1	HPCJ483
gi|11225872|emb|AX036256.1|AX036256	AX036256.1	AX036256
gi|1749761|dbj|D89872.1|D89872	D89872.1	D89872
gi|5918940|gb|AF165051.1|AF165051	AF165051.1	AF165051
gi|4753718|emb|AJ132996.1|HCV132996	AJ132996.1	HCV132996
gi|7650241|gb|AF207762.1|AF207762	AF207762.1	AF207762
gi|3098645|gb|AF054254.1|AF054254	AF054254.1	AF054254
gi|9930556|gb|AF290978.1|AF290978	AF290978.1	AF290978
gi|11559462|dbj|AB049098.1|AB049098	AB049098.1	AB049098
gi|2764397|emb|AJ000009.1|HCVPOLYP	AJ000009.1	HCVPOLYP
gi|221608|dbj|D10988.1|HPCJ8G	D10988.1	HPCJ8G
gi|3098634|gb|AF054248.1|AF054248	AF054248.1	AF054248
gi|221650|dbj|D00944.1|HPCPOLP	D00944.1	HPCPOLP
gi|306286|gb|M96362.1|HPCUNKCDS	M96362.1	HPCUNKCDS
gi|3098654|gb|AF054259.1|AF054259	AF054259.1	AF054259
gi|5918952|gb|AF165057.1|AF165057	AF165057.1	AF165057
gi|7650253|gb|AF207768.1|AF207768	AF207768.1	AF207768
gi|5918966|gb|AF165064.1|AF165064	AF165064.1	AF165064
gi|15487693|gb|AF356827.1|AF356827	AF356827.1	AF356827
gi|5738246|gb|AF176573.1|AF176573	AF176573.1	AF176573
gi|11559448|dbj|AB049091.1|AB049091	AB049091.1	AB049091
gi|21397077|gb|AF511950.1|	AF511950.1	
gi|3098638|gb|AF054250.1|AF054250	AF054250.1	AF054250
gi|6707281|gb|AF169003.1|AF169003	AF169003.1	AF169003
gi|329739|gb|L02836.1|HPCCGENOM	L02836.1	HPCCGENOM
gi|6010581|gb|AF177037.1|AF177037	AF177037.1	AF177037
gi|11559442|dbj|AB049088.1|AB049088	AB049088.1	AB049088
gi|21397076|gb|AF511949.1|	AF511949.1	
gi|1030705|dbj|D50481.1|HPCK1R2	D50481.1	HPCK1R2
gi|2176384|dbj|E08264.1|E08264	E08264.1	E08264
gi|3660725|gb|AF064490.1|AF064490	AF064490.1	AF064490
gi|2252489|emb|Y11604.1|HCV4APOLY	Y11604.1	HCV4APOLY
gi|5918942|gb|AF165052.1|AF165052	AF165052.1	AF165052
gi|2895898|gb|AF046866.1|AF046866	AF046866.1	AF046866
gi|7650243|gb|AF207763.1|AF207763	AF207763.1	AF207763
gi|11559458|dbj|AB049096.1|AB049096	AB049096.1	AB049096
gi|13122263|dbj|AB047640.1|AB047640	AB047640.1	AB047640
gi|5708574|dbj|E08263.1|E08263	E08263.1	E08263
gi|7650257|gb|AF207770.1|AF207770	AF207770.1	AF207770
gi|3098647|gb|AF054255.1|AF054255	AF054255.1	AF054255

gi|11559466|dbj|AB049100.1|AB049100	AB049100.1	AB049100
gi|1181831|gb|U45476.1|HCU45476	U45476.1	HCU45476
gi|2327070|gb|AF011751.1|AF011751	AF011751.1	AF011751
gi|3098636|gb|AF054249.1|AF054249	AF054249.1	AF054249
gi|7329210|gb|AF238486.1|AF238486	AF238486.1	AF238486
gi|221612|dbj|D11168.1|HPCJTA	D11168.1	HPCJTA
gi|960359|dbj|D63857.1|HPVHCVN	D63857.1	HPVHCVN
gi|13122273|dbj|AB047645.1|AB047645	AB047645.1	AB047645
gi|5918954|gb|AF165058.1|AF165058	AF165058.1	AF165058
gi|7650255|gb|AF207769.1|AF207769	AF207769.1	AF207769
gi|437107|gb|U01214.1|HCU01214	U01214.1	HCU01214
gi|471116|dbj|D10934.1|HPCRNA	D10934.1	HPCRNA
gi|13026028|dbj|E66593.1|E66593	E66593.1	E66593
gi|2316097|gb|AF009606.1|AF009606	AF009606.1	AF009606
gi|6707283|gb|AF169004.1|AF169004	AF169004.1	AF169004
gi|514395|dbj|D17763.1|HPCEGS	D17763.1	HPCEGS
gi|9757541|dbj|AB030907.1|AB030907	AB030907.1	AB030907
gi|7329200|gb|AF238481.1|AF238481	AF238481.1	AF238481
gi|6010583|gb|AF177038.1|AF177038	AF177038.1	AF177038
gi|2172621|dbj|E04420.1|E04420	E04420.1	E04420
gi|8926244|gb|AF271632.1|AF271632	AF271632.1	AF271632
gi|5918930|gb|AF165046.1|AF165046	AF165046.1	AF165046
gi|7650231|gb|AF207757.1|AF207757	AF207757.1	AF207757
gi|5918944|gb|AF165053.1|AF165053	AF165053.1	AF165053
gi|7650245|gb|AF207764.1|AF207764	AF207764.1	AF207764
gi|12309920|emb|AX057088.1|AX057088	AX057088.1	AX057088
gi|5918958|gb|AF165060.1|AF165060	AF165060.1	AF165060
gi|7650259|gb|AF207771.1|AF207771	AF207771.1	AF207771
gi|7341102|gb|AF208024.1|AF208024	AF208024.1	AF208024
gi|3098649|gb|AF054256.1|AF054256	AF054256.1	AF054256
gi|1944375|dbj|D85516.1|D85516	D85516.1	D85516
gi|2327072|gb|AF011752.1|AF011752	AF011752.1	AF011752
gi|221614|dbj|D11355.1|HPCJTB	D11355.1	HPCJTB
gi|13122269|dbj|AB047643.1|AB047643	AB047643.1	AB047643

TABLE II: HCV siNA AND TARGET SEQUENCES

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
GCCCCGGGAGGUCUCGUAG	1	GCCCCGGGAGGUCUCGUAG	1	CUACGAGACCUCCCGGGGC	697
UGUGGUACUGCCUGAUAGG	2	UGUGGUACUGCCUGAUAGG	2	CCUAUCAGGCAGUACCACA	698
UUGUGGUACUGCCUGAUAG	3	UUGUGGUACUGCCUGAUAG	3	CUAUCAGGCAGUACCACAA	699
CCCCGGGAGGUCUCGUAGA	4	CCCCGGGAGGUCUCGUAGA	4	UCUACGAGACCUCCCGGGG	700
GUGGUACUGCCUGAUAGGG	5	GUGGUACUGCCUGAUAGGG	5	CCCUAUCAGGCAGUACCAC	701
CUGCCUGAUAGGGUGCUUG	6	CUGCCUGAUAGGGUGCUUG	6	CAAGCACCCUAUCAGGCAG	702
CCUUGUGGUACUGCCUGAU	7	CCUUGUGGUACUGCCUGAU	7	AUCAGGCAGUACCACAAGG	703
GCGAAAGGCCUUGUGGUAC	8	GCGAAAGGCCUUGUGGUAC	8	GUACCACAAGGCCUUUCGC	704
UACUGCCUGAUAGGGUGCU	9	UACUGCCUGAUAGGGUGCU	9	AGCACCCUAUCAGGCAGUA	705
GGUACUGCCUGAUAGGGUG	10	GGUACUGCCUGAUAGGGUG	10	CACCCUAUCAGGCAGUACC	706
AAAGGCCUUGUGGUACUGC	11	AAAGGCCUUGUGGUACUGC	11	GCAGUACCACAAGGCCUUU	707
AAGGCCUUGUGGUACUGCC	12	AAGGCCUUGUGGUACUGCC	12	GGCAGUACCACAAGGCCUU	708
CUUGUGGUACUGCCUGAUA	13	CUUGUGGUACUGCCUGAUA	13	UAUCAGGCAGUACCACAAG	709
AGGCCUUGUGGUACUGCCU	14	AGGCCUUGUGGUACUGCCU	14	AGGCAGUACCACAAGGCCU	710
GUACUGCCUGAUAGGGUGC	15	GUACUGCCUGAUAGGGUGC	15	GCACCCUAUCAGGCAGUAC	711
ACUGCCUGAUAGGGUGCUU	16	ACUGCCUGAUAGGGUGCUU	16	AAGCACCCUAUCAGGCAGU	712
CUUGCGAGUGCCCCGGGAG	17	CUUGCGAGUGCCCCGGGAG	17	CUCCCGGGGCACUCGCAAG	713
CUGAUAGGGUGCUUGCGAG	18	CUGAUAGGGUGCUUGCGAG	18	CUCGCAAGCACCCUAUCAG	714
UUGCGAGUGCCCCGGGAGG	19	UUGCGAGUGCCCCGGGAGG	19	CCUCCCGGGGCACUCGCAA	715
CCUGAUAGGGUGCUUGCGA	20	CCUGAUAGGGUGCUUGCGA	20	UCGCAAGCACCCUAUCAGG	716
GGCCUUGUGGUACUGCCUG	21	GGCCUUGUGGUACUGCCUG	21	CAGGCAGUACCACAAGGCC	717
GCUUGCGAGUGCCCCGGGA	22	GCUUGCGAGUGCCCCGGGA	22	UCCCGGGGCACUCGCAAGC	718
UGCCUGAUAGGGUGCUUGC	23	UGCCUGAUAGGGUGCUUGC	23	GCAAGCACCCUAUCAGGCA	719
GAAAGGCCUUGUGGUACUG	24	GAAAGGCCUUGUGGUACUG	24	CAGUACCACAAGGCCUUUC	720
GCCUGAUAGGGUGCUUGCG	25	GCCUGAUAGGGUGCUUGCG	25	CGCAAGCACCCUAUCAGGC	721
CGAAAGGCCUUGUGGUACU	26	CGAAAGGCCUUGUGGUACU	26	AGUACCACAAGGCCUUUCG	722
GCCUUGUGGUACUGCCUGA	27	GCCUUGUGGUACUGCCUGA	27	UCAGGCAGUACCACAAGGC	723

GAGUGCCCCGGGAGGUCUC	28	GAGUGCCCCGGGAGGUCUC	28	GAGACCUCCCGGGGCACUC	724
CCCGGGAGGUCUCGUAGAC	29	CCCGGGAGGUCUCGUAGAC	29	GUCUACGAGACCUCCCGGG	725
UGCGAGUGCCCCGGGAGGU	30	UGCGAGUGCCCCGGGAGGU	30	ACCUCCCGGGGCACUCGCA	726
UGGUACUGCCUGAUAGGGU	31	UGGUACUGCCUGAUAGGGU	31	ACCCUAUCAGGCAGUACCA	727
CCGGUGAGUACACCGGAAU	32	CCGGUGAGUACACCGGAAU	32	AUUCCGGUGUACUCACCGG	728
GCGAGUGCCCCGGGAGGUC	33	GCGAGUGCCCCGGGAGGUC	33	GACCUCCCGGGGCACUCGC	729
CGAGUGCCCCGGGAGGUCU	34	CGAGUGCCCCGGGAGGUCU	34	AGACCUCCCGGGGCACUCG	730
UGCCCCGGGAGGUCUCGUA	35	UGCCCCGGGAGGUCUCGUA	35	UACGAGACCUCCCGGGGCA	731
GUGCCCCGGGAGGUCUCGU	36	GUGCCCCGGGAGGUCUCGU	36	ACGAGACCUCCCGGGGCAC	732
AGUGCCCCGGGAGGUCUCG	37	AGUGCCCCGGGAGGUCUCG	37	CGAGACCUCCCGGGGCACU	733
CCGGGAGGUCUCGUAGACC	38	CCGGGAGGUCUCGUAGACC	38	GGUCUACGAGACCUCCCGG	734
UGAUAGGGUGCUUGCGAGU	39	UGAUAGGGUGCUUGCGAGU	39	ACUCGCAAGCACCCUAUCA	735
GUGCUUGCGAGUGCCCCGG	40	GUGCUUGCGAGUGCCCCGG	40	CCGGGGCACUCGCAAGCAC	736
AUAGGGUGCUUGCGAGUGC	41	AUAGGGUGCUUGCGAGUGC	41	GCACUCGCAAGCACCCUAU	737
GGGUGCUUGCGAGUGCCCC	42	GGGUGCUUGCGAGUGCCCC	42	GGGGCACUCGCAAGCACCC	738
CGGGAGGUCUCGUAGACCG	43	CGGGAGGUCUCGUAGACCG	43	CGGUCUACGAGACCUCCCG	739
GGGAGGUCUCGUAGACCGU	44	GGGAGGUCUCGUAGACCGU	44	ACGGUCUACGAGACCUCCC	740
GAUAGGGUGCUUGCGAGUG	45	GAUAGGGUGCUUGCGAGUG	45	CACUCGCAAGCACCCUAUC	741
GGAGGUCUCGUAGACCGUG	46	GGAGGUCUCGUAGACCGUG	46	CACGGUCUACGAGACCUCC	742
AGGGUGCUUGCGAGUGCCC	47	AGGGUGCUUGCGAGUGCCC	47	GGGCACUCGCAAGCACCCU	743
UGCUUGCGAGUGCCCCGGG	48	UGCUUGCGAGUGCCCCGGG	48	CCCGGGGCACUCGCAAGCA	744
GGUGCUUGCGAGUGCCCCG	49	GGUGCUUGCGAGUGCCCCG	49	CGGGGCACUCGCAAGCACC	745
UAGGGUGCUUGCGAGUGCC	50	UAGGGUGCUUGCGAGUGCC	50	GGCACUCGCAAGCACCCUA	746
AGGUCUCGUAGACCGUGCA	51	AGGUCUCGUAGACCGUGCA	51	UGCACGGUCUACGAGACCU	747
GAGGUCUCGUAGACCGUGC	52	GAGGUCUCGUAGACCGUGC	52	GCACGGUCUACGAGACCUC	748
GGAACCGGUGAGUACACCG	53	GGAACCGGUGAGUACACCG	53	CGGUGUACUCACCGGUUCC	749
CGGAACCGGUGAGUACACC	54	CGGAACCGGUGAGUACACC	54	GGUGUACUCACCGGUUCCG	750
CGGUGAGUACACCGGAAUU	55	CGGUGAGUACACCGGAAUU	55	AAUUCCGGUGUACUCACCG	751
GCGGAACCGGUGAGUACAC	56	GCGGAACCGGUGAGUACAC	56	GUGUACUCACCGGUUCCGC	752
AACCGGUGAGUACACCGGA	57	AACCGGUGAGUACACCGGA	57	UCCGGUGUACUCACCGGUU	753
ACCGGUGAGUACACCGGAA	58	ACCGGUGAGUACACCGGAA	58	UUCCGGUGUACUCACCGGU	754
CUGCGGAACCGGUGAGUAC	59	CUGCGGAACCGGUGAGUAC	59	GUACUCACCGGUUCCGCAG	755
GUCUGCGGAACCGGUGAGU	60	GUCUGCGGAACCGGUGAGU	60	ACUCACCGGUUCCGCAGAC	756
GAACCGGUGAGUACACCGG	61	GAACCGGUGAGUACACCGG	61	CCGGUGUACUCACCGGUUC	757

UGCGGAACCGGUGAGUACA	62	UGCGGAACCGGUGAGUACA	62	UGUACUCACCGGUUCCGCA	758
UCUGCGGAACCGGUGAGUA	63	UCUGCGGAACCGGUGAGUA	63	UACUCACCGGUUCCGCAGA	759
GGGAGAGCCAUAGUGGUCU	64	GGGAGAGCCAUAGUGGUCU	64	AGACCACUAUGGCUCUCCC	760
GUGGUCUGCGGAACCGGUG	65	GUGGUCUGCGGAACCGGUG	65	CACCGGUUCCGCAGACCAC	761
GGUCUGCGGAACCGGUGAG	66	GGUCUGCGGAACCGGUGAG	66	CUCACCGGUUCCGCAGACC	762
CGGGAGAGCCAUAGUGGUC	67	CGGGAGAGCCAUAGUGGUC	67	GACCACUAUGGCUCUCCCG	763
CCGGGAGAGCCAUAGUGGU	68	CCGGGAGAGCCAUAGUGGU	68	ACCACUAUGGCUCUCCCGG	764
UGGUCUGCGGAACCGGUGA	69	UGGUCUGCGGAACCGGUGA	69	UCACCGGUUCCGCAGACCA	765
GUGAGUACACCGGAAUUGC	70	GUGAGUACACCGGAAUUGC	70	GCAAUUCCGGUGUACUCAC	766
UGAGUACACCGGAAUUGCC	71	UGAGUACACCGGAAUUGCC	71	GGCAAUUCCGGUGUACUCA	767
GGUGAGUACACCGGAAUUG	72	GGUGAGUACACCGGAAUUG	72	CAAUUCCGGUGUACUCACC	768
GAGCCAUAGUGGUCUGCGG	73	GAGCCAUAGUGGUCUGCGG	73	CCGCAGACCACUAUGGCUC	769
AGAGCCAUAGUGGUCUGCG	74	AGAGCCAUAGUGGUCUGCG	74	CGCAGACCACUAUGGCUCU	770
UAGUGGUCUGCGGAACCGG	75	UAGUGGUCUGCGGAACCGG	75	CCGGUUCCGCAGACCACUA	771
AUAGUGGUCUGCGGAACCG	76	AUAGUGGUCUGCGGAACCG	76	CGGUUCCGCAGACCACUAU	772
GAGAGCCAUAGUGGUCUGC	77	GAGAGCCAUAGUGGUCUGC	77	GCAGACCACUAUGGCUCUC	773
GCCAUAGUGGUCUGCGGAA	78	GCCAUAGUGGUCUGCGGAA	78	UUCCGCAGACCACUAUGGC	774
AGUGGUCUGCGGAACCGGU	79	AGUGGUCUGCGGAACCGGU	79	ACCGGUUCCGCAGACCACU	775
CAUAGUGGUCUGCGGAACC	80	CAUAGUGGUCUGCGGAACC	80	GGUUCCGCAGACCACUAUG	776
AGCCAUAGUGGUCUGCGGA	81	AGCCAUAGUGGUCUGCGGA	81	UCCGCAGACCACUAUGGCU	777
CCAUAGUGGUCUGCGGAAC	82	CCAUAGUGGUCUGCGGAAC	82	GUUCCGCAGACCACUAUGG	778
CCCCUCCCGGGAGAGCCAU	83	CCCCUCCCGGGAGAGCCAU	83	AUGGCUCUCCCGGGAGGGG	779
GGAGAGCCAUAGUGGUCUG	84	GGAGAGCCAUAGUGGUCUG	84	CAGACCACUAUGGCUCUCC	780
CCCGGGAGAGCCAUAGUGG	85	CCCGGGAGAGCCAUAGUGG	85	CCACUAUGGCUCUCCCGGG	781
CCCCCUCCCGGGAGAGCCA	86	CCCCCUCCCGGGAGAGCCA	86	UGGCUCUCCCGGGAGGGGG	782
UCCCGGGAGAGCCAUAGUG	87	UCCCGGGAGAGCCAUAGUG	87	CACUAUGGCUCUCCCGGGA	783
CCCCCCUCCCGGGAGAGCC	88	CCCCCCUCCCGGGAGAGCC	88	GGCUCUCCCGGGAGGGGGG	784
CCCUCCCGGGAGAGCCAUA	89	CCCUCCCGGGAGAGCCAUA	89	UAUGGCUCUCCCGGGAGGG	785
CCUCCCGGGAGAGCCAUAG	90	CCUCCCGGGAGAGCCAUAG	90	CUAUGGCUCUCCCGGGAGG	786
CUCCCGGGAGAGCCAUAGU	91	CUCCCGGGAGAGCCAUAGU	91	ACUAUGGCUCUCCCGGGAG	787
UGUUGCCGCGCAGGGGCCC	92	UGUUGCCGCGCAGGGGCCC	92	GGGCCCCUGCGCGGCAACA	788
CCCCCCCUCCCGGGAGAGC	93	CCCCCCCUCCCGGGAGAGC	93	GCUCUCCCGGGAGGGGGGG	789
CAUGGCGUUAGUAUGAGUG	94	CAUGGCGUUAGUAUGAGUG	94	CACUCAUACUAACGCCAUG	790
UAGCCAUGGCGUUAGUAUG	95	UAGCCAUGGCGUUAGUAUG	95	CAUACUAACGCCAUGGCUA	791

AGCCAUGGCGUUAGUAUGA	96	AGCCAUGGCGUUAGUAUGA	96	UCAUACUAACGCCAUGGCU	792
CCAUGGCGUUAGUAUGAGU	97	CCAUGGCGUUAGUAUGAGU	97	ACUCAUACUAACGCCAUGG	793
AUGGCGUUAGUAUGAGUGU	98	AUGGCGUUAGUAUGAGUGU	98	ACACUCAUACUAACGCCAU	794
AAGCGUCUAGCCAUGGCGU	99	AAGCGUCUAGCCAUGGCGU	99	ACGCCAUGGCUAGACGCUU	795
GUCUAGCCAUGGCGUUAGU	100	GUCUAGCCAUGGCGUUAGU	100	ACUAACGCCAUGGCUAGAC	796
AAAGCGUCUAGCCAUGGCG	101	AAAGCGUCUAGCCAUGGCG	101	CGCCAUGGCUAGACGCUUU	797
GCGUCUAGCCAUGGCGUUA	102	GCGUCUAGCCAUGGCGUUA	102	UAACGCCAUGGCUAGACGC	798
GCCAUGGCGUUAGUAUGAG	103	GCCAUGGCGUUAGUAUGAG	103	CUCAUACUAACGCCAUGGC	799
AGCGUCUAGCCAUGGCGUU	104	AGCGUCUAGCCAUGGCGUU	104	AACGCCAUGGCUAGACGCU	800
CGUCUAGCCAUGGCGUUAG	105	CGUCUAGCCAUGGCGUUAG	105	CUAACGCCAUGGCUAGACG	801
UCUAGCCAUGGCGUUAGUA	106	UCUAGCCAUGGCGUUAGUA	106	UACUAACGCCAUGGCUAGA	802
GAAAGCGUCUAGCCAUGGC	107	GAAAGCGUCUAGCCAUGGC	107	GCCAUGGCUAGACGCUUUC	803
CUAGCCAUGGCGUUAGUAU	108	CUAGCCAUGGCGUUAGUAU	108	AUACUAACGCCAUGGCUAG	804
CACUCCCCUGUGAGGAACU	109	CACUCCCCUGUGAGGAACU	109	AGUUCCUCACAGGGGAGUG	805
ACCUCAAAGAAAAACCAAA	110	ACCUCAAAGAAAAACCAAA	110	UUUGGUUUUUCUUUGAGGU	806
CGCAGAAAGCGUCUAGCCA	111	CGCAGAAAGCGUCUAGCCA	111	UGGCUAGACGCUUUCUGCG	807
GGGUAAGGUCAUCGAUACC	112	GGGUAAGGUCAUCGAUACC	112	GGUAUCGAUGACCUUACCC	808
CAGAAAGCGUCUAGCCAUG	113	CAGAAAGCGUCUAGCCAUG	113	CAUGGCUAGACGCUUUCUG	809
AAACCUCAAAGAAAAACCA	114	AAACCUCAAAGAAAAACCA	114	UGGUUUUUCUUUGAGGUUU	810
GCAGAAAGCGUCUAGCCAU	115	GCAGAAAGCGUCUAGCCAU	115	AUGGCUAGACGCUUUCUGC	811
AGAAAGCGUCUAGCCAUGG	116	AGAAAGCGUCUAGCCAUGG	116	CCAUGGCUAGACGCUUUCU	812
ACGCAGAAAGCGUCUAGCC	117	ACGCAGAAAGCGUCUAGCC	117	GGCUAGACGCUUUCUGCGU	813
AACCUCAAAGAAAAACCAA	118	AACCUCAAAGAAAAACCAA	118	UUGGUUUUUCUUUGAGGUU	814
UGGGUAAGGUCAUCGAUAC	119	UGGGUAAGGUCAUCGAUAC	119	GUAUCGAUGACCUUACCCA	815
GUAAGGUCAUCGAUACCCU	120	GUAAGGUCAUCGAUACCCU	120	AGGGUAUCGAUGACCUUAC	816
UUCACGCAGAAAGCGUCUA	121	UUCACGCAGAAAGCGUCUA	121	UAGACGCUUUCUGCGUGAA	817
GGUAAGGUCAUCGAUACCC	122	GGUAAGGUCAUCGAUACCC	122	GGGUAUCGAUGACCUUACC	818
AUCACUCCCCUGUGAGGAA	123	AUCACUCCCCUGUGAGGAA	123	UUCCUCACAGGGGAGUGAU	819
UCACUCCCCUGUGAGGAAC	124	UCACUCCCCUGUGAGGAAC	124	GUUCCUCACAGGGGAGUGA	820
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CACGCAGAAAGCGUCUAGC	127	CACGCAGAAAGCGUCUAGC	127	GCUAGACGCUUUCUGCGUG	823
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GAGGAACUACUGUCUUCAC	129	GAGGAACUACUGUCUUCAC	129	GUGAAGACAGUAGUUCCUC	825

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UGGCGUUAGUAUGAGUGUC	142	UGGCGUUAGUAUGAGUGUC	142	GACACUCAUACUAACGCCA	838
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GCGUGCCCCCGCGAGACUG	326	GCGUGCCCCCGCGAGACUG	326	CAGUCUCGCGGGGGCACGC	1022
GACCCCCCCUCCCGGGAGA	327	GACCCCCCCUCCCGGGAGA	327	UCUCCCGGGAGGGGGGGUC	1023
CGGGUCCUUUCUUGGAUCA	328	CGGGUCCUUUCUUGGAUCA	328	UGAUCCAAGAAAGGACCCG	1024
GUGCCCCCGCGAGACUGCU	329	GUGCCCCCGCGAGACUGCU	329	AGCAGUCUCGCGGGGGCAC	1025
CGUGCCCCCGCGAGACUGC	330	CGUGCCCCCGCGAGACUGC	330	GCAGUCUCGCGGGGGCACG	1026
UUCGCCGACCUCAUGGGGU	331	UUCGCCGACCUCAUGGGGU	331	ACCCCAUGAGGUCGGCGAA	1027
CGCCCACAGGACGUCAAGU	332	CGCCCACAGGACGUCAAGU	332	ACUUGACGUCCUGUGGGCG	1028
GCCCACAGGACGUCAAGUU	333	GCCCACAGGACGUCAAGUU	333	AACUUGACGUCCUGUGGGC	1029

ACCCCCCCUCCCGGGAGAG	334	ACCCCCCCUCCCGGGAGAG	334	CUCUCCCGGGAGGGGGGGU	1030
GGACCCCCCCUCCCGGGAG	335	GGACCCCCCCUCCCGGGAG	335	CUCCCGGGAGGGGGGGUCC	1031
CCGGGUCCUUUCUUGGAUC	336	CCGGGUCCUUUCUUGGAUC	336	GAUCCAAGAAAGGACCCGG	1032
CAGGACCCCCCCUCCCGGG	337	CAGGACCCCCCCUCCCGGG	337	CCCGGGAGGGGGGGUCCUG	1033
AGGACGUCAAGUUCCCGGG	338	AGGACGUCAAGUUCCCGGG	338	CCCGGGAACUUGACGUCCU	1034
AGGACCCCCCCUCCCGGGA	339	AGGACCCCCCCUCCCGGGA	339	UCCCGGGAGGGGGGGUCCU	1035
CCACAGGACGUCAAGUUCC	340	CCACAGGACGUCAAGUUCC	340	GGAACUUGACGUCCUGUGG	1036
CAGGACGUCAAGUUCCCGG	341	CAGGACGUCAAGUUCCCGG	341	CCGGGAACUUGACGUCCUG	1037
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CACAGGACGUCAAGUUCCC	343	CACAGGACGUCAAGUUCCC	343	GGGAACUUGACGUCCUGUG	1039
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GGGCUCAGCCCGGGUACCC	345	GGGCUCAGCCCGGGUACCC	345	GGGUACCCGGGCUGAGCCC	1041
CCGAGCGGUCGCAACCUCG	346	CCGAGCGGUCGCAACCUCG	346	CGAGGUUGCGACCGCUCGG	1042
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UCCGAGCGGUCGCAACCUC	349	UCCGAGCGGUCGCAACCUC	349	GAGGUUGCGACCGCUCGGA	1045
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GGUACCCUUGGCCCCUCUA	351	GGUACCCUUGGCCCCUCUA	351	UAGAGGGGCCAAGGGUACC	1047
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GGGUACCCUUGGCCCCUCU	353	GGGUACCCUUGGCCCCUCU	353	AGAGGGGCCAAGGGUACCC	1049
GGGUCCUUUCUUGGAUCAA	354	GGGUCCUUUCUUGGAUCAA	354	UUGAUCCAAGAAAGGACCC	1050
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GUGGGCAGGAUGGCUCCUG	357	GUGGGCAGGAUGGCUCCUG	357	CAGGAGCCAUCCUGCCCAC	1053
GGUGGGCAGGAUGGCUCCU	358	GGUGGGCAGGAUGGCUCCU	358	AGGAGCCAUCCUGCCCACC	1054
GUUGCUCUUUCUCUAUCUU	359	GUUGCUCUUUCUCUAUCUU	359	AAGAUAGAGAAAGAGCAAC	1055
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CCAGGACCCCCCCUCCCGG	361	CCAGGACCCCCCCUCCCGG	361	CCGGGAGGGGGGGUCCUGG	1057
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CUUCACGGAGGCUAUGACU	363	CUUCACGGAGGCUAUGACU	363	AGUCAUAGCCUCCGUGAAG	1059
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UUCACGGAGGCUAUGACUA	367	UUCACGGAGGCUAUGACUA	367	UAGUCAUAGCCUCCGUGAA	1063

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GUGCAGUGGAUGAACCGGC	372	GUGCAGUGGAUGAACCGGC	372	GCCGGUUCAUCCACUGCAC	1068
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AGCCCGGGUACCCUUGGCC	385	AGCCCGGGUACCCUUGGCC	385	GGCCAAGGGUACCCGGGCU	1081
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CUUGGAUCAACCCGCUCAA	399	CUUGGAUCAACCCGCUCAA	399	UUGAGCGGGUUGAUCCAAG	1095
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UGGAUCAACCCGCUCAAUG	413	UGGAUCAACCCGCUCAAUG	413	CAUUGAGCGGGUUGAUCCA	1109
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GCCCGGGUACCCUUGGCCC	429	GCCCGGGUACCCUUGGCCC	429	GGGCCAAGGGUACCCGGGC	1125
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UGGGUGUGCGCGCGACUAG	524	UGGGUGUGCGCGCGACUAG	524	CUAGUCGCGCGCACACCCA	1220
GGUGUGCGCGCGACUAGGA	525	GGUGUGCGCGCGACUAGGA	525	UCCUAGUCGCGCGCACACC	1221
GGGUGUGCGCGCGACUAGG	526	GGGUGUGCGCGCGACUAGG	526	CCUAGUCGCGCGCACACCC	1222
CCCCGGCGUAGGUCGCGUA	527	CCCCGGCGUAGGUCGCGUA	527	UACGCGACCUACGCCGGGG	1223
GAAGGCGACAACCUAUCCC	528	GAAGGCGACAACCUAUCCC	528	GGGAUAGGUUGUCGCCUUC	1224
CCCGGCGUAGGUCGCGUAA	529	CCCGGCGUAGGUCGCGUAA	529	UUACGCGACCUACGCCGGG	1225
AGCGGAGACGGCUGGAGCG	530	AGCGGAGACGGCUGGAGCG	530	CGCUCCAGCCGUCUCCGCU	1226
CCCCCGGCGUAGGUCGCGU	531	CCCCCGGCGUAGGUCGCGU	531	ACGCGACCUACGCCGGGGG	1227
AGGCGAAGGCGUCCACAGU	532	AGGCGAAGGCGUCCACAGU	532	ACUGUGGACGCCUUCGCCU	1228
AAGGCGAAGGCGUCCACAG	533	AAGGCGAAGGCGUCCACAG	533	CUGUGGACGCCUUCGCCUU	1229
GUUGGGUGUGCGCGCGACU	534	GUUGGGUGUGCGCGCGACU	534	AGUCGCGCGCACACCCAAC	1230
CUCAUGGGGUACAUUCCGC	535	CUCAUGGGGUACAUUCCGC	535	GCGGAAUGUACCCCAUGAG	1231
GGAAGGCGACAACCUAUCC	536	GGAAGGCGACAACCUAUCC	536	GGAUAGGUUGUCGCCUUCC	1232
GCAAGUUCCUUGCCGACGG	537	GCAAGUUCCUUGCCGACGG	537	CCGUCGGCAAGGAACUUGC	1233

UGCAGCCUCCAGGACCCCC	538	UGCAGCCUCCAGGACCCCC	538	GGGGGUCCUGGAGGCUGCA	1234
GGACUGCACGAUGCUCGUG	539	GGACUGCACGAUGCUCGUG	539	CACGAGCAUCGUGCAGUCC	1235
GAAGGCGAAGGCGUCCACA	540	GAAGGCGAAGGCGUCCACA	540	UGUGGACGCCUUCGCCUUC	1236
GCAACCUCGUGGAAGGCGA	541	GCAACCUCGUGGAAGGCGA	541	UCGCCUUCCACGAGGUUGC	1237
GACGCGGGCUGUGCUUGGU	542	GACGCGGGCUGUGCUUGGU	542	ACCAAGCACAGCCCGCGUC	1238
ACGCGGGCUGUGCUUGGUA	543	ACGCGGGCUGUGCUUGGUA	543	UACCAAGCACAGCCCGCGU	1239
GUGCAGCCUCCAGGACCCC	544	GUGCAGCCUCCAGGACCCC	544	GGGGUCCUGGAGGCUGCAC	1240
GCAGCCUCCAGGACCCCCC	545	GCAGCCUCCAGGACCCCCC	545	GGGGGGUCCUGGAGGCUGC	1241
CGCAACCUCGUGGAAGGCG	546	CGCAACCUCGUGGAAGGCG	546	CGCCUUCCACGAGGUUGCG	1242
UGUCGUGCAGCCUCCAGGA	547	UGUCGUGCAGCCUCCAGGA	547	UCCUGGAGGCUGCACGACA	1243
AUGGCUUGGGAUAUGAUGA	548	AUGGCUUGGGAUAUGAUGA	548	UCAUCAUAUCCCAAGCCAU	1244
CUUGGGAUAUGAUGAUGAA	549	CUUGGGAUAUGAUGAUGAA	549	UUCAUCAUCAUAUCCCAAG	1245
CCCUUGGCCCCUCUAUGGC	550	CCCUUGGCCCCUCUAUGGC	550	GCCAUAGAGGGGCCAAGGG	1246
UGGCUUGGGAUAUGAUGAU	551	UGGCUUGGGAUAUGAUGAU	551	AUCAUCAUAUCCCAAGCCA	1247
CUGUGCAGUGGAUGAACCG	552	CUGUGCAGUGGAUGAACCG	552	CGGUUCAUCCACUGCACAG	1248
AUGACGCGGGCUGUGCUUG	553	AUGACGCGGGCUGUGCUUG	553	CAAGCACAGCCCGCGUCAU	1249
GCUUGGGAUAUGAUGAUGA	554	GCUUGGGAUAUGAUGAUGA	554	UCAUCAUCAUAUCCCAAGC	1250
UAUGACGCGGGCUGUGCUU	555	UAUGACGCGGGCUGUGCUU	555	AAGCACAGCCCGCGUCAUA	1251
UGACGCGGGCUGUGCUUGG	556	UGACGCGGGCUGUGCUUGG	556	CCAAGCACAGCCCGCGUCA	1252
GGCUUGGGAUAUGAUGAUG	557	GGCUUGGGAUAUGAUGAUG	557	CAUCAUCAUAUCCCAAGCC	1253
UGUGCAGUGGAUGAACCGG	558	UGUGCAGUGGAUGAACCGG	558	CCGGUUCAUCCACUGCACA	1254
GCUGUGCAGUGGAUGAACC	559	GCUGUGCAGUGGAUGAACC	559	GGUUCAUCCACUGCACAGC	1255
CUCUUCAACUGGGCAGUAA	560	CUCUUCAACUGGGCAGUAA	560	UUACUGCCCAGUUGAAGAG	1256
CCUCGUGGAAGGCGACAAC	561	CCUCGUGGAAGGCGACAAC	561	GUUGUCGCCUUCCACGAGG	1257
UGUGUCACCCAGACAGUCG	562	UGUGUCACCCAGACAGUCG	562	CGACUGUCUGGGUGACACA	1258
GGCGUGAACUAUGCAACAG	563	GGCGUGAACUAUGCAACAG	563	CUGUUGCAUAGUUCACGCC	1259
CGGCGUGAACUAUGCAACA	564	CGGCGUGAACUAUGCAACA	564	UGUUGCAUAGUUCACGCCG	1260
GUGUCACCCAGACAGUCGA	565	GUGUCACCCAGACAGUCGA	565	UCGACUGUCUGGGUGACAC	1261
CCUCUUCAACUGGGCAGUA	566	CCUCUUCAACUGGGCAGUA	566	UACUGCCCAGUUGAAGAGG	1262
CGUGGAAGGCGACAACCUA	567	CGUGGAAGGCGACAACCUA	567	UAGGUUGUCGCCUUCCACG	1263
UCGUGGAAGGCGACAACCU	568	UCGUGGAAGGCGACAACCU	568	AGGUUGUCGCCUUCCACGA	1264
CGGCCUAGUUGGGGCCCCA	569	CGGCCUAGUUGGGGCCCCA	569	UGGGGCCCCAACUAGGCCG	1265
CGACUAGGAAGACUUCCGA	570	CGACUAGGAAGACUUCCGA	570	UCGGAAGUCUUCCUAGUCG	1266
UUUGGGUAAGGUCAUCGAU	571	UUUGGGUAAGGUCAUCGAU	571	AUCGAUGACCUUACCCAAA	1267

GUGGAAGGCGACAACCUAU	572	GUGGAAGGCGACAACCUAU	572	AUAGGUUGUCGCCUUCCAC	1268
ACCUCGUGGAAGGCGACAA	573	ACCUCGUGGAAGGCGACAA	573	UUGUCGCCUUCCACGAGGU	1269
GCGACUAGGAAGACUUCCG	574	GCGACUAGGAAGACUUCCG	574	CGGAAGUCUUCCUAGUCGC	1270
GUCGUGCAGCCUCCAGGAC	575	GUCGUGCAGCCUCCAGGAC	575	GUCCUGGAGGCUGCACGAC	1271
UAGGAAGACUUCCGAGCGG	576	UAGGAAGACUUCCGAGCGG	576	CCGCUCGGAAGUCUUCCUA	1272
ACGGCGUGAACUAUGCAAC	577	ACGGCGUGAACUAUGCAAC	577	GUUGCAUAGUUCACGCCGU	1273
CUCGUGGAAGGCGACAACC	578	CUCGUGGAAGGCGACAACC	578	GGUUGUCGCCUUCCACGAG	1274
GGUCGCAACCUCGUGGAAG	579	GGUCGCAACCUCGUGGAAG	579	CUUCCACGAGGUUGCGACC	1275
CGGUCGCAACCUCGUGGAA	580	CGGUCGCAACCUCGUGGAA	580	UUCCACGAGGUUGCGACCG	1276
GCGCGCGACUAGGAAGACU	581	GCGCGCGACUAGGAAGACU	581	AGUCUUCCUAGUCGCGCGC	1277
GACGGCGUGAACUAUGCAA	582	GACGGCGUGAACUAUGCAA	582	UUGCAUAGUUCACGCCGUC	1278
UAGAUCACUCCCCUGUGAG	583	UAGAUCACUCCCCUGUGAG	583	CUCACAGGGGAGUGAUCUA	1279
AGCGGUCGCAACCUCGUGG	584	AGCGGUCGCAACCUCGUGG	584	CCACGAGGUUGCGACCGCU	1280
UGGAAGGCGACAACCUAUC	585	UGGAAGGCGACAACCUAUC	585	GAUAGGUUGUCGCCUUCCA	1281
CGCGCGACUAGGAAGACUU	586	CGCGCGACUAGGAAGACUU	586	AAGUCUUCCUAGUCGCGCG	1282
CUAGGAAGACUUCCGAGCG	587	CUAGGAAGACUUCCGAGCG	587	CGCUCGGAAGUCUUCCUAG	1283
GUGCGCGCGACUAGGAAGA	588	GUGCGCGCGACUAGGAAGA	588	UCUUCCUAGUCGCGCGCAC	1284
AGAUCACUCCCCUGUGAGG	589	AGAUCACUCCCCUGUGAGG	589	CCUCACAGGGGAGUGAUCU	1285
UGCGCGCGACUAGGAAGAC	590	UGCGCGCGACUAGGAAGAC	590	GUCUUCCUAGUCGCGCGCA	1286
AUAGAUCACUCCCCUGUGA	591	AUAGAUCACUCCCCUGUGA	591	UCACAGGGGAGUGAUCUAU	1287
GAGCGGUCGCAACCUCGUG	592	GAGCGGUCGCAACCUCGUG	592	CACGAGGUUGCGACCGCUC	1288
CACGAACGACUGCUCCAAC	593	CACGAACGACUGCUCCAAC	593	GUUGGAGCAGUCGUUCGUG	1289
GGCAAGUUCCUUGCCGACG	594	GGCAAGUUCCUUGCCGACG	594	CGUCGGCAAGGAACUUGCC	1290
UCGUGCAGCCUCCAGGACC	595	UCGUGCAGCCUCCAGGACC	595	GGUCCUGGAGGCUGCACGA	1291
GUCACGAACGACUGCUCCA	596	GUCACGAACGACUGCUCCA	596	UGGAGCAGUCGUUCGUGAC	1292
GCGGUCGCAACCUCGUGGA	597	GCGGUCGCAACCUCGUGGA	597	UCCACGAGGUUGCGACCGC	1293
GCGCGACUAGGAAGACUUC	598	GCGCGACUAGGAAGACUUC	598	GAAGUCUUCCUAGUCGCGC	1294
GCUAUGACGCGGGCUGUGC	599	GCUAUGACGCGGGCUGUGC	599	GCACAGCCCGCGUCAUAGC	1295
UCACGAACGACUGCUCCAA	600	UCACGAACGACUGCUCCAA	600	UUGGAGCAGUCGUUCGUGA	1296
UCGCAACCUCGUGGAAGGC	601	UCGCAACCUCGUGGAAGGC	601	GCCUUCCACGAGGUUGCGA	1297
CGUGCAGCCUCCAGGACCC	602	CGUGCAGCCUCCAGGACCC	602	GGGUCCUGGAGGCUGCACG	1298
GUCGCAACCUCGUGGAAGG	603	GUCGCAACCUCGUGGAAGG	603	CCUUCCACGAGGUUGCGAC	1299
ACUAGGAAGACUUCCGAGC	604	ACUAGGAAGACUUCCGAGC	604	GCUCGGAAGUCUUCCUAGU	1300
CGCGACUAGGAAGACUUCC	605	CGCGACUAGGAAGACUUCC	605	GGAAGUCUUCCUAGUCGCG	1301

UGGGCGAAGCACAUGUGGA	606	UGGGCGAAGCACAUGUGGA	606	UCCACAUGUGCUUCGCCCA	1302
CCUUGCCUACUAUUCCAUG	607	CCUUGCCUACUAUUCCAUG	607	CAUGGAAUAGUAGGCAAGG	1303
GCCUCAGGAAACUUGGGGU	608	GCCUCAGGAAACUUGGGGU	608	ACCCCAAGUUUCCUGAGGC	1304
UGCUAUGACGCGGGCUGUG	609	UGCUAUGACGCGGGCUGUG	609	CACAGCCCGCGUCAUAGCA	1305
UCGUGCUCGCCACCGCUAC	610	UCGUGCUCGCCACCGCUAC	610	GUAGCGGUGGCGAGCACGA	1306
UGCCUCAGGAAACUUGGGG	611	UGCCUCAGGAAACUUGGGG	611	CCCCAAGUUUCCUGAGGCA	1307
UGUCUCGUGCCCGACCCCG	612	UGUCUCGUGCCCGACCCCG	612	CGGGGUCGGGCACGAGACA	1308
UGUGGCGGCAGGAGAUGGG	613	UGUGGCGGCAGGAGAUGGG	613	CCCAUCUCCUGCCGCCACA	1309
GUCGUGCUCGCCACCGCUA	614	GUCGUGCUCGCCACCGCUA	614	UAGCGGUGGCGAGCACGAC	1310
GAUUUCCACUACGUGACGG	615	GAUUUCCACUACGUGACGG	615	CCGUCACGUAGUGGAAAUC	1311
GGGCCUUGCCUACUAUUCC	616	GGGCCUUGCCUACUAUUCC	616	GGAAUAGUAGGCAAGGCCC	1312
GCCUUGCCUACUAUUCCAU	617	GCCUUGCCUACUAUUCCAU	617	AUGGAAUAGUAGGCAAGGC	1313
GACUAGGAAGACUUCCGAG	618	GACUAGGAAGACUUCCGAG	618	CUCGGAAGUCUUCCUAGUC	1314
GCGGGGGAGACAUAUAUCA	619	GCGGGGGAGACAUAUAUCA	619	UGAUAUAUGUCUCCCCCGC	1315
CGAGCGGUCGCAACCUCGU	620	CGAGCGGUCGCAACCUCGU	620	ACGAGGUUGCGACCGCUCG	1316
GGCCUUGCCUACUAUUCCA	621	GGCCUUGCCUACUAUUCCA	621	UGGAAUAGUAGGCAAGGCC	1317
AUUUCCACUACGUGACGGG	622	AUUUCCACUACGUGACGGG	622	CCCGUCACGUAGUGGAAAU	1318
GGACGUCAAGUUCCCGGGC	623	GGACGUCAAGUUCCCGGGC	623	GCCCGGGAACUUGACGUCC	1319
GAGUGCUAUGACGCGGGCU	624	GAGUGCUAUGACGCGGGCU	624	AGCCCGCGUCAUAGCACUC	1320
GACGUCAAGUUCCCGGGCG	625	GACGUCAAGUUCCCGGGCG	625	CGCCCGGGAACUUGACGUC	1321
UCAGCGACGGGUCUUGGUC	626	UCAGCGACGGGUCUUGGUC	626	GACCAAGACCCGUCGCUGA	1322
UCAAGUUCCCGGGCGGUGG	627	UCAAGUUCCCGGGCGGUGG	627	CCACCGCCCGGGAACUUGA	1323
UCAAGGAGAUGAAGGCGAA	628	UCAAGGAGAUGAAGGCGAA	628	UUCGCCUUCAUCUCCUUGA	1324
CCUAUCCCCAAGGCUCGCC	629	CCUAUCCCCAAGGCUCGCC	629	GGCGAGCCUUGGGGAUAGG	1325
CUUGACCUACCUCAGAUCA	630	CUUGACCUACCUCAGAUCA	630	UGAUCUGAGGUAGGUCAAG	1326
UUUCCACUACGUGACGGGC	631	UUUCCACUACGUGACGGGC	631	GCCCGUCACGUAGUGGAAA	1327
AGUGCUAUGACGCGGGCUG	632	AGUGCUAUGACGCGGGCUG	632	CAGCCCGCGUCAUAGCACU	1328
ACGUCAAGUUCCCGGGCGG	633	ACGUCAAGUUCCCGGGCGG	633	CCGCCCGGGAACUUGACGU	1329
UCUGGAGACAUCGGGCCAG	634	UCUGGAGACAUCGGGCCAG	634	CUGGCCCGAUGUCUCCAGA	1330
GGGCGAAGCACAUGUGGAA	635	GGGCGAAGCACAUGUGGAA	635	UUCCACAUGUGCUUCGCCC	1331
UUGACCUACCUCAGAUCAU	636	UUGACCUACCUCAGAUCAU	636	AUGAUCUGAGGUAGGUCAA	1332
CCAAGCGGAGACGGCUGGA	637	CCAAGCGGAGACGGCUGGA	637	UCCAGCCGUCUCCGCUUGG	1333
ACCAAGCGGAGACGGCUGG	638	ACCAAGCGGAGACGGCUGG	638	CCAGCCGUCUCCGCUUGGU	1334
GGGUGGCUUCAUGCCUCAG	639	GGGUGGCUUCAUGCCUCAG	639	CUGAGGCAUGAAGCCACCC	1335

GUCAAGUUCCCGGGCGGUG	640	GUCAAGUUCCCGGGCGGUG	640	CACCGCCCGGGAACUUGAC	1336
CUCAAGGAGAUGAAGGCGA	641	CUCAAGGAGAUGAAGGCGA	641	UCGCCUUCAUCUCCUUGAG	1337
GACCAAGCGGAGACGGCUG	642	GACCAAGCGGAGACGGCUG	642	CAGCCGUCUCCGCUUGGUC	1338
UCCAGGUCGGGCUCAACCA	643	UCCAGGUCGGGCUCAACCA	643	UGGUUGAGCCCGACCUGGA	1339
CUCUUUCUCUAUCUUCCUC	644	CUCUUUCUCUAUCUUCCUC	644	GAGGAAGAUAGAGAAAGAG	1340
GUCUGGAGACAUCGGGCCA	645	GUCUGGAGACAUCGGGCCA	645	UGGCCCGAUGUCUCCAGAC	1341
GUUGUGACUUGGCCCCCGA	646	GUUGUGACUUGGCCCCCGA	646	UCGGGGGCCAAGUCACAAC	1342
AGACCUGGCUCCAGUCCAA	647	AGACCUGGCUCCAGUCCAA	647	UUGGACUGGAGCCAGGUCU	1343
CUUGCCUACUAUUCCAUGG	648	CUUGCCUACUAUUCCAUGG	648	CCAUGGAAUAGUAGGCAAG	1344
CCCGGUUGCUCUUUCUCUA	649	CCCGGUUGCUCUUUCUCUA	649	UAGAGAAAGAGCAACCGGG	1345
CUUUCUCUAUCUUCCUCUU	650	CUUUCUCUAUCUUCCUCUU	650	AAGAGGAAGAUAGAGAAAG	1346
AGGGUGGCUUCAUGCCUCA	651	AGGGUGGCUUCAUGCCUCA	651	UGAGGCAUGAAGCCACCCU	1347
AAGACCUGGCUCCAGUCCA	652	AAGACCUGGCUCCAGUCCA	652	UGGACUGGAGCCAGGUCUU	1348
CCGGUUGCUCUUUCUCUAU	653	CCGGUUGCUCUUUCUCUAU	653	AUAGAGAAAGAGCAACCGG	1349
CGGUUGCUCUUUCUCUAUC	654	CGGUUGCUCUUUCUCUAUC	654	GAUAGAGAAAGAGCAACCG	1350
UGGGGGAUUUCCACUACGU	655	UGGGGGAUUUCCACUACGU	655	ACGUAGUGGAAAUCCCCCA	1351
AUGUCACGAACGACUGCUC	656	AUGUCACGAACGACUGCUC	656	GAGCAGUCGUUCGUGACAU	1352
GGCCUAGUUGGGGCCCCAC	657	GGCCUAGUUGGGGCCCCAC	657	GUGGGGCCCCAACUAGGCC	1353
UGGACCAAGCGGAGACGGC	658	UGGACCAAGCGGAGACGGC	658	GCCGUCUCCGCUUGGUCCA	1354
UUCCAGGUCGGGCUCAACC	659	UUCCAGGUCGGGCUCAACC	659	GGUUGAGCCCGACCUGGAA	1355
AGCGGGUCGAGUUCCUGGU	660	AGCGGGUCGAGUUCCUGGU	660	ACCAGGAACUCGACCCGCU	1356
CAAGGAGAUGAAGGCGAAG	661	CAAGGAGAUGAAGGCGAAG	661	CUUCGCCUUCAUCUCCUUG	1357
CAUGUCACGAACGACUGCU	662	CAUGUCACGAACGACUGCU	662	AGCAGUCGUUCGUGACAUG	1358
CAGCGGGUCGAGUUCCUGG	663	CAGCGGGUCGAGUUCCUGG	663	CCAGGAACUCGACCCGCUG	1359
UUCCACUACGUGACGGGCA	664	UUCCACUACGUGACGGGCA	664	UGCCCGUCACGUAGUGGAA	1360
UAGGGUGGCUUCAUGCCUC	665	UAGGGUGGCUUCAUGCCUC	665	GAGGCAUGAAGCCACCCUA	1361
UCCAGGACUGCACGAUGCU	666	UCCAGGACUGCACGAUGCU	666	AGCAUCGUGCAGUCCUGGA	1362
UCCACUACGUGACGGGCAU	667	UCCACUACGUGACGGGCAU	667	AUGCCCGUCACGUAGUGGA	1363
AAUAGGGUGGCUUCAUGCC	668	AAUAGGGUGGCUUCAUGCC	668	GGCAUGAAGCCACCCUAUU	1364
GUCUUCACGGAGGCUAUGA	669	GUCUUCACGGAGGCUAUGA	669	UCAUAGCCUCCGUGAAGAC	1365
AUAGGGUGGCUUCAUGCCU	670	AUAGGGUGGCUUCAUGCCU	670	AGGCAUGAAGCCACCCUAU	1366
UCUUCACGGAGGCUAUGAC	671	UCUUCACGGAGGCUAUGAC	671	GUCAUAGCCUCCGUGAAGA	1367
AUGCCUCAGGAAACUUGGG	672	AUGCCUCAGGAAACUUGGG	672	CCCAAGUUUCCUGAGGCAU	1368
ACCGGGACGUGCUCAAGGA	673	ACCGGGACGUGCUCAAGGA	673	UCCUUGAGCACGUCCCGGU	1369

GGGGCUGUGCAGUGGAUGA	674	GGGGCUGUGCAGUGGAUGA	674	UCAUCCACUGCACAGCCCC	1370
AAGCUCCAGGACUGCACGA	675	AAGCUCCAGGACUGCACGA	675	UCGUGCAGUCCUGGAGCUU	1371
GCUCCAGGACUGCACGAUG	676	GCUCCAGGACUGCACGAUG	676	CAUCGUGCAGUCCUGGAGC	1372
UACCGGGACGUGCUCAAGG	677	UACCGGGACGUGCUCAAGG	677	CCUUGAGCACGUCCCGGUA	1373
GGGCUGUGCAGUGGAUGAA	678	GGGCUGUGCAGUGGAUGAA	678	UUCAUCCACUGCACAGCCC	1374
CGUCAAGUUCCCGGGCGGU	679	CGUCAAGUUCCCGGGCGGU	679	ACCGCCCGGGAACUUGACG	1375
UCAAUAGGGUGGCUUCAUG	680	UCAAUAGGGUGGCUUCAUG	680	CAUGAAGCCACCCUAUUGA	1376
AGUCUUCACGGAGGCUAUG	681	AGUCUUCACGGAGGCUAUG	681	CAUAGCCUCCGUGAAGACU	1377
GGACCAAGCGGAGACGGCU	682	GGACCAAGCGGAGACGGCU	682	AGCCGUCUCCGCUUGGUCC	1378
GGCUCCAGUCCAAGCUCCU	683	GGCUCCAGUCCAAGCUCCU	683	AGGAGCUUGGACUGGAGCC	1379
GGCUGUGCAGUGGAUGAAC	684	GGCUGUGCAGUGGAUGAAC	684	GUUCAUCCACUGCACAGCC	1380
CUCCAGGACUGCACGAUGC	685	CUCCAGGACUGCACGAUGC	685	GCAUCGUGCAGUCCUGGAG	1381
GAGUCUUCACGGAGGCUAU	686	GAGUCUUCACGGAGGCUAU	686	AUAGCCUCCGUGAAGACUC	1382
UGGCUCCAGUCCAAGCUCC	687	UGGCUCCAGUCCAAGCUCC	687	GGAGCUUGGACUGGAGCCA	1383
GGGGAUUUCCACUACGUGA	688	GGGGAUUUCCACUACGUGA	688	UCACGUAGUGGAAAUCCCC	1384
CAUGCCUCAGGAAACUUGG	689	CAUGCCUCAGGAAACUUGG	689	CCAAGUUUCCUGAGGCAUG	1385
AUCAAUAGGGUGGCUUCAU	690	AUCAAUAGGGUGGCUUCAU	690	AUGAAGCCACCCUAUUGAU	1386
GCGGGCCUUGCCUACUAUU	691	GCGGGCCUUGCCUACUAUU	691	AAUAGUAGGCAAGGCCCGC	1387
CCGGGACGUGCUCAAGGAG	692	CCGGGACGUGCUCAAGGAG	692	CUCCUUGAGCACGUCCCGG	1388
CCAUGGUGGGGAACUGGGC	693	CCAUGGUGGGGAACUGGGC	693	GCCCAGUUCCCCACCAUGG	1389
CAAUAGGGUGGCUUCAUGC	694	CAAUAGGGUGGCUUCAUGC	694	GCAUGAAGCCACCCUAUUG	1390
AGCUCCAGGACUGCACGAU	695	AGCUCCAGGACUGCACGAU	695	AUCGUGCAGUCCUGGAGCU	1391
CGGGCCUUGCCUACUAUUC	696	CGGGCCUUGCCUACUAUUC	696	GAAUAGUAGGCAAGGCCCG	1392

The 3'-ends of the Upper sequence and the Lower sequence of the siNA construct can include an overhang sequence, for example about 1, 2, 3, or 4 nucleotides in length, preferably 2 nucleotides in length, wherein the overhanging sequence of the lower sequence is optionally complementary to a portion of the target sequence. The upper and lower sequences in the Table can further comprise a chemical modification having Formulae I-VII, such as exemplary siNA constructs shown in Figures 4 and 5, or having modifications described in Table IV or any combination thereof.

TABLE III: HCV Synthetic Modified siNA Constructs

Target Pos	Target	Seq ID	Cmpd#	Aliases	Sequence	Seq ID
183	GGUCCUUUCUUGGAUCAACCCGC	1393	25237	HCV IRES Loop IIIb (Heptazyme site) as siNA str1 (sense)	B GGUCCUUUCUUGGAUCAACCC B	1467
183	GGUCCUUUCUUGGAUCAACCCGC	1393	25238	HCV IRES Loop IIIb (Heptazyme site) as siNA str2 (antisense)	B GGGUUGAUCCAAGAAAGGACC B	1468
183	GGUCCUUUCUUGGAUCAACCCGC	1393	25251	HCV IRES Loop IIIb (Heptazyme site) as siNA str1 (sense) Inverted Control	B CCCAACUAGGUUCUUUCCUGG B	1469
183	GGUCCUUUCUUGGAUCAACCCGC	1393	25252	HCV IRES Loop IIIb (Heptazyme site) as siNA str1 (sense) Inverted Control Compliment	B CCAGGAAAGAACCUAGUUGGG B	1470
183	GGUCCUUUCUUGGAUCAACCCGC	1393	25814	HCV IRES Loop IIIb (Heptazyme site) as siNA str1 (sense)+2U overhang	GGUCCUUUCUUGGAUCAACCCUU	1471
183	GGUCCUUUCUUGGAUCAACCCGC	1393	25815	HCV IRES Loop IIIb (Heptazyme site) as siNA str2 (antisense) +2U overhang	GGGUUGAUCCAAGAAAGGACCUU	1472
183	GGUCCUUUCUUGGAUCAACCCGC	1393	25834	HCV IRES Loop IIIb (Heptazyme site) as siNA str1 (sense)+2U overhang	BGGUCCUUUCUUGGAUCAACCCUUB	1473
183	GGUCCUUUCUUGGAUCAACCCGC	1393	25835	HCV IRES Loop IIIb (Heptazyme site) as siNA str2 (antisense) +2U overhang	BGGGUUGAUCCAAGAAAGGACCUUB	1474
325	UGCCCCGGGAGGUCUCGUAGACC	1394	28415	HCVa:325U21 sense TT siNA	CCCCGGGAGGUCUCGUAGATT	1475
162	CGGAACCGGUGAGUACACC	54	28416	HCVa:162U21 sense TT siNA	CGGAACCGGUGAGUACACCTT	1476
324	GCCCCGGGAGGUCUCGUAG	1	28417	HCVa:324U21 sense TT siNA	GCCCCGGGAGGUCUCGUAGTT	1477
163	GGAACCGGUGAGUACACCG	53	28418	HCVa:163U21 sense TT siNA	GGAACCGGUGAGUACACCGTT	1478
294	GUGGUACUGCCUGAUAGGG	5	28419	HCVa:294U21 sense TT siNA	GUGGUACUGCCUGAUAGGGTT	1479
293	UGUGGUACUGCCUGAUAGG	2	28420	HCVa:293U21 sense TT siNA	UGUGGUACUGCCUGAUAGGTT	1480
292	UUGUGGUACUGCCUGAUAG	3	28421	HCVa:292U21 sense TT siNA	UUGUGGUACUGCCUGAUAGTT	1481
325	UGCCCCGGGAGGUCUCGUAGACC	1394	28422	HCVa :343L21 antisense TT siNA (325C)	UCUACGAGACCUCCCGGGGTT	1482
162	CGGAACCGGUGAGUACACC	54	28423	HCVa :180L21 antisense TT siNA (162C)	GGUGUACUCACCGGUUCCGTT	1483
324	GCCCCGGGAGGUCUCGUAG	1	28424	HCVa :342L21 antisense TT siNA (324C)	CUACGAGACCUCCCGGGGCTT	1484
163	GGAACCGGUGAGUACACCG	53	28425	HCVa :181L21 antisense TT siNA (163C)	CGGUGUACUCACCGGUUCCTT	1485

294	GUGGUACUGCCUGAUAGGG	5	28426	HCVa :312L21 antisense TT siNA (294C)	CCCUAUCAGGCAGUACCACTT	1486
293	UGUGGUACUGCCUGAUAGG	2	28427	HCVa :311L21 antisense TT siNA (293C)	CCUAUCAGGCAGUACCACATT	1487
292	UUGUGGUACUGCCUGAUAG	3	28428	HCVa :310L21 antisense TT siNA (292C)	CUAUCAGGCAGUACCACAATT	1488
325	UGCCCCGGGAGGUCUCGUAGACC	1394	28429	HCVa:325U21 sense TT siNA inv	TTAGAUGCUCUGGAGGGCCCC	1489
162	CGGAACCGGUGAGUACACC	54	28430	HCVa:162U21 sense TT siNA inv	TTCCACAUGAGUGGCCAAGGC	1490
324	GCCCCGGGAGGUCUCGUAG	1	28431	HCVa:324U21 sense TT siNA inv	TTGAUGCUCUGGAGGGCCCCG	1491
163	GGAACCGGUGAGUACACCG	53	28432	HCVa:163U21 sense TT siNA inv	TTGCCACAUGAGUGGCCAAGG	1492
294	GUGGUACUGCCUGAUAGGG	5	28433	HCVa:294U21 sense TT siNA inv	TTGGGAUAGUCCGUCAUGGUG	1493
293	UGUGGUACUGCCUGAUAGG	2	28434	HCVa:293U21 sense TT siNA inv	TTGGAUAGUCCGUCAUGGUGU	1494
292	UUGUGGUACUGCCUGAUAG	3	28435	HCVa:292U21 sense TT siNA inv	TTGAUAGUCCGUCAUGGUGUU	1495
325	UGCCCCGGGAGGUCUCGUAGACC	1394	28436	HCVa :343L21 antisense TT siNA (325C) inv	TTGGGGCCCUCCAGAGCAUCU	1496
162	CGGAACCGGUGAGUACACC	54	28437	HCVa :180L21 antisense TT siNA (162C) inv	TTGCCUUGGCCACUCAUGUGG	1497
324	GCCCCGGGAGGUCUCGUAG	1	28438	HCVa :342L21 antisense TT siNA (324C) inv	TTCGGGGCCCUCCAGAGCAUC	1498
163	GGAACCGGUGAGUACACCG	53	28439	HCVa :181L21 antisense TT siNA (163C) inv	TTCCUUGGCCACUCAUGUGGC	1499
294	GUGGUACUGCCUGAUAGGG	5	28440	HCVa :312L21 antisense TT siNA (294C) inv	TTCACCAUGACGGACUAUCCC	1500
293	UGUGGUACUGCCUGAUAGG	2	28441	HCVa :311L21 antisense TT siNA (293C) inv	TTACACCAUGACGGACUAUCC	1501
292	UUGUGGUACUGCCUGAUAG	3	28442	HCVa :310L21 antisense TT siNA (292C) inv	TTAACACCAUGACGGACUAUC	1502
162	UGCGGAACCGGUGAGUACACCGG	1395	29573	HCVa:162U21 sense siNA	CGGAACCGGUGAGUACACCGG	1503
163	GCGGAACCGGUGAGUACACCGGA	1396	29574	HCVa:163U21 sense siNA	GGAACCGGUGAGUACACCGGA	1504
292	CCUUGUGGUACUGCCUGAUAGGG	1397	29575	HCVa:292U21 sense siNA	UUGUGGUACUGCCUGAUAGGG	1505
293	CUUGUGGUACUGCCUGAUAGGGU	1398	29576	HCVa:293U21 sense siNA	UGUGGUACUGCCUGAUAGGGU	1506
294	UUGUGGUACUGCCUGAUAGGGUG	1399	29577	HCVa:294U21 sense siNA	GUGGUACUGCCUGAUAGGGUG	1507
324	GUGCCCCGGGAGGUCUCGUAGAC	1400	29578	HCVa:324U21 sense siNA	GCCCCGGGAGGUCUCGUAGAC	1508
325	UGCCCCGGGAGGUCUCGUAGACC	1394	29579	HCVa:325U21 sense siNA	CCCCGGGAGGUCUCGUAGACC	1509
162	UGCGGAACCGGUGAGUACACCGG	1395	29580	HCVa :180L21 antisense siNA (162C)	GGUGUACUCACCGGUUCCGCA	1510
163	GCGGAACCGGUGAGUACACCGGA	1396	29581	HCVa :181L21 antisense siNA (163C)	CGGUGUACUCACCGGUUCCGC	1511
292	CCUUGUGGUACUGCCUGAUAGGG	1397	29582	HCVa :310L21 antisense siNA (292C)	CUAUCAGGCAGUACCACAAGG	1512

293	CUUGUGGUACUGCCUGAUAGGGU	1398	29583	HCVa :311L21 antisense siNA (293C)	CCUAUCAGGCAGUACCACAAG	1513
294	UUGUGGUACUGCCUGAUAGGGUG	1399	29584	HCVa :312L21 antisense siNA (294C)	CCCUAUCAGGCAGUACCACAA	1514
324	GUGCCCCGGGAGGUCUCGUAGAC	1400	29585	HCVa :342L21 antisense siNA (324C)	CUACGAGACCUCCCGGGGCAC	1515
325	UGCCCCGGGAGGUCUCGUAGACC	1394	29586	HCVa :343L21 antisense siNA (325C)	UCUACGAGACCUCCCGGGGCA	1516
162	UGCGGAACCGGUGAGUACACCGG	1395	29587	HCVa:162U21 sense siNA inv	GGCCACAUGAGUGGCCAAGGC	1517
163	GCGGAACCGGUGAGUACACCGGA	1396	29588	HCVa:163U21 sense siNA inv	AGGCCACAUGAGUGGCCAAGG	1518
292	CCUUGUGGUACUGCCUGAUAGGG	1397	29589	HCVa:292U21 sense siNA inv	GGGAUAGUCCGUCAUGGUGUU	1519
293	CUUGUGGUACUGCCUGAUAGGGU	1398	29590	HCVa:293U21 sense siNA inv	UGGGAUAGUCCGUCAUGGUGU	1520
294	UUGUGGUACUGCCUGAUAGGGUG	1399	29591	HCVa:294U21 sense siNA inv	GUGGGAUAGUCCGUCAUGGUG	1521
324	GUGCCCCGGGAGGUCUCGUAGAC	1400	29592	HCVa:324U21 sense siNA inv	CAGAUGCUCUGGAGGGCCCCG	1522
325	UGCCCCGGGAGGUCUCGUAGACC	1394	29593	HCVa:325U21 sense siNA inv	CCAGAUGCUCUGGAGGGCCCC	1523
162	UGCGGAACCGGUGAGUACACCGG	1395	29594	HCVa :180L21 antisense siNA (162C) inv	ACGCCUUGGCCACUCAUGUGG	1524
163	GCGGAACCGGUGAGUACACCGGA	1396	29595	HCVa :181L21 antisense siNA (163C) inv	CGCCUUGGCCACUCAUGUGGC	1525
292	CCUUGUGGUACUGCCUGAUAGGG	1397	29596	HCVa :310L21 antisense siNA (292C) inv	GGAACACCAUGACGGACUAUC	1526
293	CUUGUGGUACUGCCUGAUAGGGU	1398	29597	HCVa :311L21 antisense siNA (293C) inv	GAACACCAUGACGGACUAUCC	1527
294	UUGUGGUACUGCCUGAUAGGGUG	1399	29598	HCVa :312L21 antisense siNA (294C) inv	AACACCAUGACGGACUAUCCC	1528
324	GUGCCCCGGGAGGUCUCGUAGAC	1400	29599	HCVa :342L21 antisense siNA (324C) inv	CACGGGGCCCUCCAGAGCAUC	1529
325	UGCCCCGGGAGGUCUCGUAGACC	1394	29600	HCVa :343L21 antisense siNA (325C) inv	ACGGGGCCCUCCAGAGCAUCU	1530
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30051	HCVa:325U21 sense siNA 5 5' P=S + 3' univ. base 2 + 5'/3' invAba	BCsCsCsCsGsGGAGGUCUCGUAGAXXB	1531
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30052	HCVa:325U21 sense siNA inv 5 5' P=S + 3' univ. base 2 + 5'/3' invAba	BAsGsAsUsGsCUCUGGAGGGCCCCXXB	1532
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30053	HCVa:343L21 antisense siNA (325C) 5 5' P=S + 3' univ. base 2 + 3' invAba	UsCsUsAsCsGAGACCUCCCGGGGXXB	1533
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30054	HCVa:343L21 antisense siNA (325C) inv 5 5' P=S + 3' univ. base 2 + 3' invAba	GsGsGsGsCsCCUCCAGAGCAUCUXXB	1534
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30055	HCVa:325U21 sense siNA all Y P=S + 3' univ. base 2 + 5'/3' invAba	BCsCsCsCsGGGAGGUsCsUsCsGUsAGAXXB	1535
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30056	HCVa:325U21 sense siNA inv all Y	BAGAUsGCsUsCsUsGGAGGGCsCsCsCsXXB	1536

				P=S + 3' univ. base 2 + 5'/3' invAba		
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30057	HCVa:343L21 antisense siNA (325C) all Y P=S + 3' univ. base 2 + 3' invAba	UsCsUsACsGAGACsCsUsCsCsCsGGGGXXB	1537
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30058	HCVa:343L21 antisense siNA (325C) inv all Y P=S + 3' univ. base 2 + 3' invAba	GGGGCsCsCsUsCsCsAGAGCsAUsCsUsXXB	1538
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30059	HCVa:325U21 sense siNA 4/3 P=S ends + all Y-2'F + 3' univ. base 2 + 5'/3' invAba	BcscscscsGGGAGGucucGuAsGsAsXXB	1539
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30060	HCVa:325U21 sense siNA inv 4/3 P=S ends + all Y-2'F + 3' univ. base 2 + 5'/3' invAba	BAsGsAsusGcucuGGAGGGccscscsXXB	1540
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30170	HCVa:325U21 sense siNA all Y-2'F + 3' univ. base 2 + 5'/3' invAba	B ccccGGGAGGucucGuAGAXX B	1541
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30171	HCVa:325U21 sense siNA inv all Y-2'F + 3' univ. base 2 + 5'/3' invAba	B AGAuGcucuGGAGGGccccXX B	1542
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30172	HCVa:343L21 antisense siNA (325C) all Y P=S + 3' univ. base 2 + 5'/3' invAba	B UsCsUsACsGAGACsCsUsCsCsCsGGGGXX B	1543
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30173	HCVa:343L19 antisense siNA (325C) all Y-2'F	ucuAcGAGAccuccccGGGG	1544
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30175	HCVa :343L21 antisense siNA (325C) all Y-2'F + 3' univ. Base 2	ucuAcGAGAccuccccGGGGXX	1545
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30176	HCVa :343L21 antisense siNA (325C) inv all Y-2'F + 3' univ. Base 2	GGGGccccuccAGAGcAucuXX	1546
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30177	HCVa :343L21 antisense siNA (325C) all Y-2'F + 3' univ. Base 2 + 5'/3' iB	B ucuAcGAGAccuccccGGGGXX B	1547
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30178	HCVa:325U21 sense siNA all Y P=S + 3' univ. base 2 + 3' invAba	CsCsCsCsGGGAGGUsCsUsCsGUsAGAXX B	1548
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30417	HCVa:325U21 sense siNA w/iB	CCCCGGGAGGUCUCGUAGACC B	1549
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30418	HCVa:325U21 sense siNA w/iB	B CCCCGGGAGGUCUCGUAGACC B	1550
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30419	HCVa :343L21 antisense siNA (325C) w/iB	UCUACGAGACCUCCCGGGGCA B	1551
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30420	HCVa :343L21 antisense siNA (325C) w/iB	B UCUACGAGACCUCCCGGGGCA B	1552
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30561	HCVa :325U21 sense siNA Y-2'Ome (stab06) + 5'/3' invAba	B ccccGGGAGGucucGuAGATT B	1553
325	UGCCCCGGGAGGUCUCGUAGACC	1394	30562	HCVa :343L21 antisense siNA (325C) Y-2'F, R-2'Ome + TsT	ucuAcGAGAccuccccGGGGTsT	1554
153	AUAGUGGUCUGCGGAACCGGUGA	1401	30649	HCVa:153U21 sense siNA stab07	B *AGuGGucuGcGGAAccGGuTT* B	1555

159	GUCUGCGGAACCGGUGAGUACAC	1402	30650	HCVa:159U21 sense siNA stab07	B cuGcGGAAccGGuGAGuAcTT B	1556
291	GCCUUGUGGUACUGCCUGAUAGG	1403	30651	HCVa:291U21 sense siNA stab07	B cuuGuGGuAcuGccuGAuATT B	1557
295	UGUGGUACUGCCUGAUAGGGUGC	1404	30652	HCVa:295U21 sense siNA stab07	B uGGuAcuGccuGAuAGGGuTT B	1558
296	GUGGUACUGCCUGAUAGGGUGCU	1405	30653	HCVa:296U21 sense siNA stab07	B GGuAcuGccuGAuAGGGuGTT B	1559
297	UGGUACUGCCUGAUAGGGUGCUU	1406	30654	HCVa:297U21 sense siNA stab07	B GuAcuGccuGAuAGGGuGcTT B	1560
298	GGUACUGCCUGAUAGGGUGCUUG	1407	30655	HCVa:298U21 sense siNA stab07	B uAcuGccuGAuAGGGuGcuTT B	1561
300	UACUGCCUGAUAGGGUGCUUGCG	1408	30656	HCVa:300U21 sense siNA stab07	B cuGccuGAuAGGGuGcuuGTT B	1562
301	ACUGCCUGAUAGGGUGCUUGCGA	1409	30657	HCVa:301U21 sense siNA stab07	B uGccuGAuAGGGuGcuuGcTT B	1563
303	UGCCUGAUAGGGUGCUUGCGAGU	1410	30658	HCVa:303U21 sense siNA stab07	B ccuGAuAGGGuGcuuGcGATT B	1564
306	CUGAUAGGGUGCUUGCGAGUGCC	1411	30659	HCVa:306U21 sense siNA stab07	B GAuAGGGuGcuuGcGAGuGTT B	1565
324	GUGCCCCGGGAGGUCUCGUAGAC	1400	30660	HCVa:324U21 sense siNA stab07	B GccccGGGAGGucucGuAGTT B	1566
153	AUAGUGGUCUGCGGAACCGGUGA	1401	30661	HCVa :171L21 antisense siNA (153C) stab08	AccGGuuccGcAGAccAcuTsT	1567
159	GUCUGCGGAACCGGUGAGUACAC	1402	30662	HCVa :177L21 antisense siNA (159C) stab08	GuAcucAccGGuuccGcAGTsT	1568
291	GCCUUGUGGUACUGCCUGAUAGG	1403	30663	HCVa :309L21 antisense siNA (291C) stab08	uAucAGGcAGuAccAcAAGTsT	1569
295	UGUGGUACUGCCUGAUAGGGUGC	1404	30664	HCVa :313L21 antisense siNA (295C) stab08	AcccuAucAGGcAGuAccATsT	1570
296	GUGGUACUGCCUGAUAGGGUGCU	1405	30665	HCVa :314L21 antisense siNA (296C) stab08	cAcccuAucAGGcAGuAccTsT	1571
297	UGGUACUGCCUGAUAGGGUGCUU	1406	30666	HCVa :315L21 antisense siNA (297C) stab08	GcAcccuAucAGGcAGuAcTsT	1572
298	GGUACUGCCUGAUAGGGUGCUUG	1407	30667	HCVa :316L21 antisense siNA (298C) stab08	AGcAcccuAucAGGcAGuATsT	1573
300	UACUGCCUGAUAGGGUGCUUGCG	1408	30668	HCVa :318L21 antisense siNA (300C) stab08	cAAGcAcccuAucAGGcAGTsT	1574
301	ACUGCCUGAUAGGGUGCUUGCGA	1409	30669	HCVa :319L21 antisense siNA (301C) stab08	GcAAGcAcccuAucAGGcATsT	1575
303	UGCCUGAUAGGGUGCUUGCGAGU	1410	30670	HCVa :321L21 antisense siNA (303C) stab08	ucGcAAGcAcccuAucAGGTsT	1576
306	CUGAUAGGGUGCUUGCGAGUGCC	1411	30671	HCVa :324L21 antisense siNA (306C) stab08	cAcucGcAAGcAcccuAucTsT	1577
324	GUGCCCCGGGAGGUCUCGUAGAC	1400	30672	HCVa :342L21 antisense siNA (324C) stab08	cuAcGAGAccucccGGGGcTsT	1578
153	AUAGUGGUCUGCGGAACCGGUGA	1401	30673	HCVa:153U21 sense siNA stab07 inv	B uGGccAAGGcGucuGGuGATT B	1579
159	GUCUGCGGAACCGGUGAGUACAC	1402	30674	HCVa:159U21 sense siNA stab07 inv	B cAuGAGuGGccAAGGcGucTT B	1580
291	GCCUUGUGGUACUGCCUGAUAGG	1403	30675	HCVa:291U21 sense siNA stab07 inv	B AuAGuccGucAuGGuGuucTT B	1581
295	UGUGGUACUGCCUGAUAGGGUGC	1404	30676	HCVa:295U21 sense siNA stab07 inv	B uGGGAuAGuccGucAuGGuTT B	1582
296	GUGGUACUGCCUGAUAGGGUGCU	1405	30677	HCVa:296U21 sense siNA stab07 inv	B GuGGGAuAGuccGucAuGGTT B	1583

297	UGGUACUGCCUGAUAGGGUGCUU	1406	30678	HCVa:297U21 sense siNA stab07 inv	B cGuGGGAuAGuccGucAuGTT B	1584
298	GGUACUGCCUGAUAGGGUGCUUG	1407	30679	HCVa:298U21 sense siNA stab07 inv	B ucGuGGGAuAGuccGucAuTT B	1585
300	UACUGCCUGAUAGGGUGCUUGCG	1408	30680	HCVa:300U21 sense siNA stab07 inv	B GuucGuGGGAuAGuccGucTT B	1586
301	ACUGCCUGAUAGGGUGCUUGCGA	1409	30681	HCVa:301U21 sense siNA stab07 inv	B cGuucGuGGGAuAGuccGuTT B	1587
303	UGCCUGAUAGGGUGCUUGCGAGU	1410	30682	HCVa:303U21 sense siNA stab07 inv	B AGcGuucGuGGGAuAGuccTT B	1588
306	CUGAUAGGGUGCUUGCGAGUGCC	1411	30683	HCVa:306U21 sense siNA stab07 inv	B GuGAGcGuucGuGGGAuAGTT B	1589
324	GUGCCCCGGGAGGUCUCGUAGAC	1400	30684	HCVa:324U21 sense siNA stab07 inv	B GAuGcucuGGAGGGccccGTT B	1590
153	AUAGUGGUCUGCGGAACCGGUGA	1401	30685	HCVa:171L21 antisense siNA (153C) stab08 inv	ucAccAGAcGccuuGGccATsT	1591
159	GUCUGCGGAACCGGUGAGUACAC	1402	30686	HCVa:177L21 antisense siNA (159C) stab08 inv	GacGccuuGGccAcucAuGTsT	1592
291	GCCUUGUGGUACUGCCUGAUAGG	1403	30687	HCVa:309L21 antisense siNA (291C) stab08 inv	GAAcAccAuGAcGGAcuAuTsT	1593
295	UGUGGUACUGCCUGAUAGGGUGC	1404	30688	HCVa:313L21 antisense siNA (295C) stab08 inv	AccAuGAcGGAcuAucccATsT	1594
296	GUGGUACUGCCUGAUAGGGUGCU	1405	30689	HCVa:314L21 antisense siNA (296C) stab08 inv	ccAuGAcGGAcuAucccAcTsT	1595
297	UGGUACUGCCUGAUAGGGUGCUU	1406	30690	HCVa:315L21 antisense siNA (297C) stab08 inv	cAuGAcGGAcuAucccAcGTsT	1596
298	GGUACUGCCUGAUAGGGUGCUUG	1407	30691	HCVa:316L21 antisense siNA (298C) stab08 inv	AuGAcGGAcuAucccAcGATsT	1597
300	UACUGCCUGAUAGGGUGCUUGCG	1408	30692	HCVa:318L21 antisense siNA (300C) stab08 inv	GAcGGAcuAucccAcGAAcTsT	1598
301	ACUGCCUGAUAGGGUGCUUGCGA	1409	30693	HCVa:319L21 antisense siNA (301C) stab08 inv	AcGGAcuAucccAcGAAcGTsT	1599
303	UGCCUGAUAGGGUGCUUGCGAGU	1410	30694	HCVa:321L21 antisense siNA (303C) stab08 inv	GGAcuAucccAcGAAcGcuTsT	1600
306	CUGAUAGGGUGCUUGCGAGUGCC	1411	30695	HCVa:324L21 antisense siNA (306C) stab08 inv	cuAucccAcGAAcGcucAcTsT	1601
324	GUGCCCCGGGAGGUCUCGUAGAC	1400	30696	HCVa:342L21 antisense siNA (324C) stab08 inv	cGGGGcccuccAGAGcAucTsT	1602
325	UGCCCCGGGAGGUCUCGUAGACC	1394	31340	HCVa:325U21 sense siNA stab04	B ccccGGGAGGucucGuAGATT B	1603
325	UGCCCCGGGAGGUCUCGUAGACC	1394	31341	HCVa:325U21 sense siNA inv stab04	B AGAuGcucuGGAGGGccccTT B	1604
325	UGCCCCGGGAGGUCUCGUAGACC	1394	31342	HCVa :343L21 antisense siNA (325C) stab05	ucuAcGAGAccucccGGGGTsT	1605
325	UGCCCCGGGAGGUCUCGUAGACC	1394	31343	HCVa:343L21 antisense siNA (325C) inv stab05	GGGGcccuccAGAGcAucuTsT	1606
325	UGCCCCGGGAGGUCUCGUAGACC	1394	31344	HCVa:325U21 sense siNA stab07	B ccccGGGAGGucucGuAGATT B	1607
325	UGCCCCGGGAGGUCUCGUAGACC	1394	31345	HCVa:325U21 sense siNA inv stab07	B AGAuGcucuGGAGGGccccTT B	1608
325	UGCCCCGGGAGGUCUCGUAGACC	1394	31346	HCVa:343L21 antisense siNA (325C) inv stab08	GGGGcccuccAGAGcAucuTsT	1609

325	UGCCCCGGGAGGUCUCGUAGACC	1394	31347	HCVa :343L21 antisense siNA (325C) stab11	ucuAcGAGAccucccGGGGTsT	1610
325	UGCCCCGGGAGGUCUCGUAGACC	1394	31348	HCVa:343L21 antisense siNA (325C) inv stab11	*GGGG*cccucc*AGAG*c*A*ucuTsT	1611
153	AUAGUGGUCUGCGGAACCGGUGA	1401	31453	HCVa:153U21 sense siNA stab04	B AGuGGucuGcGGAAccGGuTT B	1612
159	GUCUGCGGAACCGGUGAGUACAC	1402	31454	HCVa:159U21 sense siNA stab04	B cuGcGGAAccGGuGAGuAcTT B	1613
287	AAAGGCCUUGUGGUACUGCCUGA	1412	31455	HCVa:287U21 sense siNA stab04	B AGGccuuGuGGuAcuGccuTT B	1614
291	GCCUUGUGGUACUGCCUGAUAGG	1403	31456	HCVa:291U21 sense siNA stab04	B cuuGuGGuAcuGccuGAuATT B	1615
295	UGUGGUACUGCCUGAUAGGGUGC	1404	31457	HCVa:295U21 sense siNA stab04	B uGGuAcuGccuGAuAGGGuTT B	1616
296	GUGGUACUGCCUGAUAGGGUGCU	1405	31458	HCVa:296U21 sense siNA stab04	B GGuAcuGccuGAuAGGGuGTT B	1617
297	UGGUACUGCCUGAUAGGGUGCUU	1406	31459	HCVa:297U21 sense siNA stab04	B GuAcuGccuGAuAGGGuGcTT B	1618
298	GGUACUGCCUGAUAGGGUGCUUG	1407	31460	HCVa:298U21 sense siNA stab04	B uAcuGccuGAuAGGGuGcuTT B	1619
300	UACUGCCUGAUAGGGUGCUUGCG	1408	31461	HCVa:300U21 sense siNA stab04	B cuGccuGAuAGGGuGcuuGTT B	1620
301	ACUGCCUGAUAGGGUGCUUGCGA	1409	31462	HCVa:301U21 sense siNA stab04	B uGccuGAuAGGGuGcuuGcTT B	1621
303	UGCCUGAUAGGGUGCUUGCGAGU	1410	31463	HCVa:303U21 sense siNA stab04	B ccuGAuAGGGuGcuuGcGATT B	1622
306	CUGAUAGGGUGCUUGCGAGUGCC	1411	31464	HCVa:306U21 sense siNA stab04	B GAuAGGGuGcuuGcGAGuGTT B	1623
153	AUAGUGGUCUGCGGAACCGGUGA	1401	31465	HCVa :171L21 antisense siNA (153C) stab05	AccGGuuccGcAGAccAcuTsT	1624
159	GUCUGCGGAACCGGUGAGUACAC	1402	31466	HCVa :177L21 antisense siNA (159C) stab05	GuAcucAccGGuuccGcAGTsT	1625
287	AAAGGCCUUGUGGUACUGCCUGA	1412	31467	HCVa :305L21 antisense siNA (287C) stab05	AGGcAGuAccAcAAGGccuTsT	1626
291	GCCUUGUGGUACUGCCUGAUAGG	1403	31468	HCVa :309L21 antisense siNA (291C) stab05	uAucAGGcAGuAccAcAAGTsT	1627
295	UGUGGUACUGCCUGAUAGGGUGC	1404	31469	HCVa :313L21 antisense siNA (295C) stab05	AcccuAucAGGcAGuAccATsT	1628
296	GUGGUACUGCCUGAUAGGGUGCU	1405	31470	HCVa :314L21 antisense siNA (296C) stab05	cAcccuAucAGGcAGuAccTsT	1629
297	UGGUACUGCCUGAUAGGGUGCUU	1406	31471	HCVa :315L21 antisense siNA (297C) stab05	GcAcccuAucAGGcAGuAcTsT	1630
298	GGUACUGCCUGAUAGGGUGCUUG	1407	31472	HCVa :316L21 antisense siNA (298C) stab05	AGcAcccuAucAGGcAGuATsT	1631
300	UACUGCCUGAUAGGGUGCUUGCG	1408	31473	HCVa :318L21 antisense siNA (300C) stab05	cAAGcAcccuAucAGGcAGTsT	1632
301	ACUGCCUGAUAGGGUGCUUGCGA	1409	31474	HCVa :319L21 antisense siNA (301C) stab05	GcAAGcAcccuAucAGGcATsT	1633
303	UGCCUGAUAGGGUGCUUGCGAGU	1410	31475	HCVa :321L21 antisense siNA (303C) stab05	ucGcAAGcAcccuAucAGGTsT	1634
306	CUGAUAGGGUGCUUGCGAGUGCC	1411	31476	HCVa :324L21 antisense siNA (306C) stab05	cAcucGcAAGcAcccuAucTsT	1635

153	AUAGUGGUCUGCGGAACCGGUGA	1401	31477	HCVa:153U21 sense siNA inv stab04	B uGGccAAGGcGucuGGuGATT B	1636
159	GUCUGCGGAACCGGUGAGUACAC	1402	31478	HCVa:159U21 sense siNA inv stab04	B cAuGAGuGGccAAGGcGucTT B	1637
287	AAAGGCCUUGUGGUACUGCCUGA	1412	31479	HCVa:287U21 sense siNA inv stab04	B uccGucAuGGuGuuccGGATT B	1638
291	GCCUUGUGGUACUGCCUGAUAGG	1403	31480	HCVa:291U21 sense siNA inv stab04	B AuAGuccGucAuGGuGuucTT B	1639
295	UGUGGUACUGCCUGAUAGGGUGC	1404	31481	HCVa:295U21 sense siNA inv stab04	B uGGGAuAGuccGucAuGGuTT B	1640
296	GUGGUACUGCCUGAUAGGGUGCU	1405	31482	HCVa:296U21 sense siNA inv stab04	B GuGGGAuAGuccGucAuGGTT B	1641
297	UGGUACUGCCUGAUAGGGUGCUU	1406	31483	HCVa:297U21 sense siNA inv stab04	B cGuGGGAuAGuccGucAuGTT B	1642
298	GGUACUGCCUGAUAGGGUGCUUG	1407	31484	HCVa:298U21 sense siNA inv stab04	B ucGuGGGAuAGuccGucAuTT B	1643
300	UACUGCCUGAUAGGGUGCUUGCG	1408	31485	HCVa:300U21 sense siNA inv stab04	B GuucGuGGGAuAGuccGucTT B	1644
301	ACUGCCUGAUAGGGUGCUUGCGA	1409	31486	HCVa:301U21 sense siNA inv stab04	B cGuucGuGGGAuAGuccGuTT B	1645
303	UGCCUGAUAGGGUGCUUGCGAGU	1410	31487	HCVa:303U21 sense siNA inv stab04	B AGcGuucGuGGGAuAGuccTT B	1646
306	CUGAUAGGGUGCUUGCGAGUGCC	1411	31488	HCVa:306U21 sense siNA inv stab04	B GuGAGcGuucGuGGGAuAGTT B	1647
153	AUAGUGGUCUGCGGAACCGGUGA	1401	31489	HCVa:171L21 antisense siNA (153C) inv stab05	ucAccAGAcGccuuGGccATsT	1648
159	GUCUGCGGAACCGGUGAGUACAC	1402	31490	HCVa:177L21 antisense siNA (159C) inv stab05	GacGccuuGGccAcucAuGTsT	1649
287	AAAGGCCUUGUGGUACUGCCUGA	1412	31491	HCVa:305L21 antisense siNA (287C) inv stab05	uccGGAAcAccAuGAcGGATsT	1650
291	GCCUUGUGGUACUGCCUGAUAGG	1403	31492	HCVa:309L21 antisense siNA (291C) inv stab05	GAAcAccAuGAcGGAcuAuTsT	1651
295	UGUGGUACUGCCUGAUAGGGUGC	1404	31493	HCVa:313L21 antisense siNA (295C) inv stab05	AccAuGAcGGAcuAucccATsT	1652
296	GUGGUACUGCCUGAUAGGGUGCU	1405	31494	HCVa:314L21 antisense siNA (296C) inv stab05	ccAuGAcGGAcuAucccAcTsT	1653
297	UGGUACUGCCUGAUAGGGUGCUU	1406	31495	HCVa:315L21 antisense siNA (297C) inv stab05	cAuGAcGGAcuAucccAcGTsT	1654
298	GGUACUGCCUGAUAGGGUGCUUG	1407	31496	HCVa:316L21 antisense siNA (298C) inv stab05	AuGAcGGAcuAucccAcGATsT	1655
300	UACUGCCUGAUAGGGUGCUUGCG	1408	31497	HCVa:318L21 antisense siNA (300C) inv stab05	GAcGGAcuAucccAcGAAcTsT	1656
301	ACUGCCUGAUAGGGUGCUUGCGA	1409	31498	HCVa:319L21 antisense siNA (301C) inv stab05	AcGGAcuAucccAcGAAcGTsT	1657
303	UGCCUGAUAGGGUGCUUGCGAGU	1410	31499	HCVa:321L21 antisense siNA (303C) inv stab05	GGAcuAucccAcGAAcGcuTsT	1658
306	CUGAUAGGGUGCUUGCGAGUGCC	1411	31500	HCVa:324L21 antisense siNA (306C) inv stab05	cuAucccAcGAAcGcucAcTsT	1659
190	GGGUCCUUUCUUGGAUCAACCCG	1413	31659	HCVb:190U21 sense siNA stab04	B GuccuuucuuGGAucAAccTT B	1660
191	GGUCCUUUCUUGGAUCAACCCGC	1393	31660	HCVb:191U21 sense siNA stab04	B uccuuucuuGGAucAAcccTT B	1661
189	CGGGUCCUUUCUUGGAUCAACCC	1414	31661	HCVb:189U21 sense siNA stab04	B GGuccuuucuuGGAucAAcTT B	1662
186	GACCGGGUCCUUUCUUGGAUCAA	1415	31662	HCVb:186U21 sense siNA stab04	B ccGGGuccuuucuuGGAucTT B	1663

190	GGGUCCUUUCUUGGAUCAACCCG	1413	31663	HCVb:208L21 antisense siNA (190C) stab05	GGuuGAuccAAGAAAGGAcTsT	1664
191	GGUCCUUUCUUGGAUCAACCCGC	1393	31664	HCVb:209L21 antisense siNA (191C) stab05	GGGuuGAuccAAGAAAGGATsT	1665
189	CGGGUCCUUUCUUGGAUCAACCC	1414	31665	HCVb:207L21 antisense siNA (189C) stab05	GuuGAuccAAGAAAGGAccTsT	1666
186	GACCGGGUCCUUUCUUGGAUCAA	1415	31666	HCVb:204L21 antisense siNA (186C) stab05	GAuccAAGAAAGGAcccGGTsT	1667
190	GGGUCCUUUCUUGGAUCAACCCG	1413	31667	HCVb:190U21 sense siNA inv stab04	B ccAAcuAGGuucuuuccuGTT B	1668
191	GGUCCUUUCUUGGAUCAACCCGC	1393	31668	HCVb:191U21 sense siNA inv stab04	B cccAAcuAGGuucuuuccuTT B	1669
189	CGGGUCCUUUCUUGGAUCAACCC	1414	31669	HCVb:189U21 sense siNA inv stab04	B cAAcuAGGuucuuuccuGGTT B	1670
186	GACCGGGUCCUUUCUUGGAUCAA	1415	31670	HCVb:186U21 sense siNA inv stab04	B cuAGGuucuuuccuGGGccTT B	1671
190	GGGUCCUUUCUUGGAUCAACCCG	1413	31671	HCVb:208L21 antisense siNA (190C) inv stab05	cAGGAAAGAAccuAGuuGGTsT	1672
191	GGUCCUUUCUUGGAUCAACCCGC	1393	31672	HCVb:209L21 antisense siNA (191C) inv stab05	AGGAAAGAAccuAGuuGGGTsT	1673
189	CGGGUCCUUUCUUGGAUCAACCC	1414	31673	HCVb:207L21 antisense siNA (189C) inv stab05	ccAGGAAAGAAccuAGuuGTsT	1674
186	GACCGGGUCCUUUCUUGGAUCAA	1415	31674	HCVb:204L21 antisense siNA (186C) inv stab05	GGcccAGGAAAGAAccuAGTsT	1675
326	GCCCCGGGAGGUCUCGUAGACCG	1416	31702	HCVa:326U21 sense siNA stab07	B cccGGGAGGucucGuAGAcTT B	1676
327	CCCCGGGAGGUCUCGUAGACCGU	1417	31703	HCVa:327U21 sense siNA stab07	B ccGGGAGGucucGuAGAccTT B	1677
328	CCCGGGAGGUCUCGUAGACCGUG	1418	31704	HCVa:328U21 sense siNA stab07	B cGGGAGGucucGuAGAccGTT B	1678
329	CCGGGAGGUCUCGUAGACCGUGC	1419	31705	HCVa:329U21 sense siNA stab07	B GGGAGGucucGuAGAccGuTT B	1679
326	GCCCCGGGAGGUCUCGUAGACCG	1416	31706	HCVa :344L21 antisense siNA (326C) stab08	GucuAcGAGAccucccGGGTsT	1680
327	CCCCGGGAGGUCUCGUAGACCGU	1417	31707	HCVa :345L21 antisense siNA (327C) stab08	GGucuAcGAGAccucccGGTsT	1681
328	CCCGGGAGGUCUCGUAGACCGUG	1418	31708	HCVa :346L21 antisense siNA (328C) stab08	cGGucuAcGAGAccucccGTsT	1682
329	CCGGGAGGUCUCGUAGACCGUGC	1419	31709	HCVa :347L21 antisense siNA (329C) stab08	AcGGucuAcGAGAccucccTsT	1683
326	GCCCCGGGAGGUCUCGUAGACCG	1416	31710	HCVa:326U21 sense siNA inv stab07	B cAGAuGcucuGGAGGGcccTT B	1684
327	CCCCGGGAGGUCUCGUAGACCGU	1417	31711	HCVa:327U21 sense siNA inv stab07	B ccAGAuGcucuGGAGGGccTT B	1685
328	CCCGGGAGGUCUCGUAGACCGUG	1418	31712	HCVa:328U21 sense siNA inv stab07	B GccAGAuGcucuGGAGGGcTT B	1686
329	CCGGGAGGUCUCGUAGACCGUGC	1419	31713	HCVa:329U21 sense siNA inv stab07	B uGccAGAuGcucuGGAGGGTT B	1687
326	GCCCCGGGAGGUCUCGUAGACCG	1416	31714	HCVa:344L21 antisense siNA (326C) inv stab08	GGGcccuccAGAGcAucuGTsT	1688
327	CCCCGGGAGGUCUCGUAGACCGU	1417	31715	HCVa:345L21 antisense siNA (327C) inv stab08	GGcccuccAGAGcAucuGGTsT	1689

328	CCCGGGAGGUCUCGUAGACCGUG	1418	31716	HCVa:346L21 antisense siNA (328C) inv stab08	GcccuccAGAGcAucuGGcTsT	1690
329	CCGGGAGGUCUCGUAGACCGUGC	1419	31717	HCVa:347L21 antisense siNA (329C) inv stab08	cccuccAGAGcAucuGGcATsT	1691
291	GCCUUGUGGUACUGCCUGAUAGG	1403	31762	HCVa:291U21 sense siNA stab08	cuuGuGGuAcuGccuGAuATsT	1692
295	UGUGGUACUGCCUGAUAGGGUGC	1404	31763	HCVa:295U21 sense siNA stab08	uGGuAcuGccuGAuAGGGuTsT	1693
325	UGCCCCGGGAGGUCUCGUAGACC	1394	31764	HCVa:325U21 sense siNA stab08	ccccGGGAGGucucGuAGATsT	1694
291	GCCUUGUGGUACUGCCUGAUAGG	1403	31765	HCVa:291U21 sense siNA inv stab08	AuAGuccGucAuGGuGuucTsT	1695
295	UGUGGUACUGCCUGAUAGGGUGC	1404	31766	HCVa:295U21 sense siNA inv stab08	uGGGAuAGuccGucAuGGuTsT	1696
325	UGCCCCGGGAGGUCUCGUAGACC	1394	31767	HCVa:325U21 sense siNA inv stab08	AGAuGcucuGGAGGGccccTsT	1697
327	CCCCGGGAGGUCUCGUAGACCGU	1417	31928	HCVa:327U21 sense siNA stab08	ccGGGAGGucucGuAGAccTsT	1698
327	CCCCGGGAGGUCUCGUAGACCGU	1417	31929	HCVa:327U21 sense siNA inv stab08	ccAGAuGcucuGGAGGGccTsT	1699
328	CCCGGGAGGUCUCGUAGACCGUG	1418	31930	HCVa:328U21 sense siNA stab08	cGGGAGGucucGuAGAccGTsT	1700
328	CCCGGGAGGUCUCGUAGACCGUG	1418	31931	HCVa:328U21 sense siNA inv stab08	GccAGAuGcucuGGAGGGcTsT	1701
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32007	HCVa:327U21 sense siNA stab08 + 5' abasic	B ccGGGAGGucucGuAGAccTsT	1702
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32008	HCVa:327U21 sense siNA stab08 + 3' abasic	ccGGGAGGucucGuAGAccTsT B	1703
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32009	HCVa:327U21 sense siNA stab08 + 5' & 3' abasic	B ccGGGAGGucucGuAGAccTsT B	1704
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32174	HCVa :327 siNA 3'-classI 10bp	UCUCGUAGACCUU GGUCUACGAGACCUCCCGGTT	1705
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32175	HCVa :327 siNA 3'-classI 8bp	UCGUAGACCUU GGUCUACGAGACCUCCCGGTT	1706
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32176	HCVa :327 siNA 3'-classI 6bp	GUAGACCUU GGUCUACGAGACCUCCCGGTT	1707
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32177	HCVa :327 siNA 3'-classI 4bp	AGACCUU GGUCUACGAGACCUCCCGGTT	1708
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32178	HCVa :327 siNA 5'-classI 10bp	GGUCUACGAGACCUCCCGGUU CCGGGAGGUCU	1709
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32179	HCVa :327 siNA 5'-classI 8bp	GGUCUACGAGACCUCCCGGUU CCGGGAGGU	1710
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32180	HCVa :327 siNA 5'-classI 6bp	GGUCUACGAGACCUCCCGGUU CCGGGAG	1711
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32181	HCVa :327 siNA 5'-classI 4bp	GGUCUACGAGACCUCCCGGUU CCGGG	1712
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32182	HCVa :327 siNA 3'-gaaa 10bp	CUCGUAGACC GAAA GGUCUACGAGACCUCCCGGTT	1713
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32183	HCVa :327 siNA 3'-gaaa 8bp	CGUAGACC GAAA GGUCUACGAGACCUCCCGGTT	1714
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32184	HCVa :327 siNA 3'-gaaa 6bp	UAGACC GAAA GGUCUACGAGACCUCCCGGTT	1715
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32185	HCVa :327 siNA 3'-gaaa 4bp	GACC GAAA GGUCUACGAGACCUCCCGGTT	1716
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32186	HCVa :327 siNA 5'-gaaa 10bp	GGUCUACGAGACCUCCCGGUU GAAA	1717

					CCGGGAGGUC	
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32187	HCVa :327 siNA 5'-gaaa 8bp	GGUCUACGAGACCUCCCGGUU GAAA CCGGGAGG	1718
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32188	HCVa :327 siNA 5'-gaaa 6bp	GGUCUACGAGACCUCCCGGUU GAAA CCGGGA	1719
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32189	HCVa :327 siNA 5'-gaaa 4bp	GGUCUACGAGACCUCCCGGUU GAAA CCGG	1720
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32190	HCVa:327 siNA 3'-uuuguguag 10bp	CGUAGACCUU UUUGUGUAG GGUCUACGAGACCUCCCGGTT	1721
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32191	HCVa:327 siNA 3'-uuuguguag 8bp	UAGACCUU UUUGUGUAG GGUCUACGAGACCUCCCGGTT	1722
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32192	HCVa:327 siNA 3'-uuuguguag 6bp	GACCUU UUUGUGUAG GGUCUACGAGACCUCCCGGTT	1723
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32193	HCVa:327 siNA 3'-uuuguguag 4bp	CCUU UUUGUGUAG GGUCUACGAGACCUCCCGGTT	1724
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32194	HCVa:327 siNA 5'-uuuguguag 10bp	GGUCUACGAGACCUCCCGGUU UUUGUGUAG CCGGGAGGUC	1725
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32195	HCVa:327 siNA 5'-uuuguguag 8bp	GGUCUACGAGACCUCCCGGUU UUUGUGUAG CCGGGAGG	1726
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32196	HCVa:327 siNA 5'-uuuguguag 6bp	GGUCUACGAGACCUCCCGGUU UUUGUGUAG CCGGGA	1727
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32197	HCVa:327 siNA 5'-uuuguguag 4bp	GGUCUACGAGACCUCCCGGUU UUUGUGUAG CCGG	1728
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32198	HCVa:345L21 antisense (327C) stab05 siNA	GGucuAcGAGAccucccGGTsT	1729
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32199	HCVa :345L21 antisense (327C) stab05 5'p siNA	pGGucuAcGAGAccucccGGTsT	1730
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32200	HCVa :345L21 antisense (327C) stab05 5'ps siNA	sGGucuAcGAGAccucccGGTsT	1731
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32201	HCVa:345L21 antisense (327C) stab00 siNA	GGUCUACGAGACCUCCCGGTT	1732
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32202	HCVa :345L21 antisense (327C) v1 5'p siNA	pGGUCUACGAGACCUCCCGGTT	1733
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32203	HCVa :345L21 antisense (327C) v1 5'ps siNA	sGGUCUACGAGACCUCCCGGTT	1734
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32204	HCVa :345L21 antisense (327C) v2 5'p siNA	pGGUCUACGAGACCUCCCGGGGTT	1735
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32205	HCVa :345L21 antisense (327C) v3 5'p siNA	pGGUCUACGAGACCUCCCGG UCUCGUA u B	1736
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32206	HCVa :345L21 antisense (327C) v4 5'p siNA	pGGUCUACGAGACCUCCCGG AGGUCUCGUA uu B	1737
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32207	HCVa :345L21 antisense (327C) v5 5'p siNA	pGGUCUACGAGACCUCCCGGTT UCUCGUA u B	1738

327	CCCCGGGAGGUCUCGUAGACCGU	1417	32208	HCVa :345L21 antisense (327C) v6 5'p siNA	pGGUCUACGAGACCUCCCGGTT AGGUCUCGUA u B	1739
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32501	HCVa:327U21 sense siNA stab04	B ccGGGAGGucucGuAGAccTT B	1740
325	UGCCCCGGGAGGUCUCGUAGACC	1394	32502	HCVa:325U21 sense siNA stab09	B CCCCGGGAGGUCUCGUAGATT B	1741
326	GCCCCGGGAGGUCUCGUAGACCG	1416	32503	HCVa:326U21 sense siNA stab09	B CCCGGGAGGUCUCGUAGACTT B	1742
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32504	HCVa:327U21 sense siNA stab09	B CCGGGAGGUCUCGUAGACCTT B	1743
328	CCCGGGAGGUCUCGUAGACCGUG	1418	32505	HCVa:328U21 sense siNA stab09	B CGGGAGGUCUCGUAGACCGTT B	1744
329	CCGGGAGGUCUCGUAGACCGUGC	1419	32506	HCVa:329U21 sense siNA stab09	B GGGAGGUCUCGUAGACCGUTT B	1745
325	UGCCCCGGGAGGUCUCGUAGACC	1394	32507	HCVa :343L21 antisense siNA (325C) stab10	UCUACGAGACCUCCCGGGGTsT	1746
326	GCCCCGGGAGGUCUCGUAGACCG	1416	32508	HCVa :344L21 antisense siNA (326C) stab10	GUCUACGAGACCUCCCGGGTsT	1747
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32509	HCVa :345L21 antisense siNA (327C) stab10	GGUCUACGAGACCUCCCGGTsT	1748
328	CCCGGGAGGUCUCGUAGACCGUG	1418	32510	HCVa :346L21 antisense siNA (328C) stab10	CGGUCUACGAGACCUCCCGTsT	1749
329	CCGGGAGGUCUCGUAGACCGUGC	1419	32511	HCVa :347L21 antisense siNA (329C) stab10	ACGGUCUACGAGACCUCCCTsT	1750
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32512	HCVa:327U21 sense siNA inv stab04	B ccAGAuGcucuGGAGGGccTT B	1751
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32513	HCVa:345L21 antisense siNA (327C) inv stab05	GGcccuccAGAGcAucuGGTsT	1752
325	UGCCCCGGGAGGUCUCGUAGACC	1394	32514	HCVa:325U21 sense siNA inv stab09	B AGAUGCUCUGGAGGGCCCCTT B	1753
326	GCCCCGGGAGGUCUCGUAGACCG	1416	32515	HCVa:326U21 sense siNA inv stab09	B CAGAUGCUCUGGAGGGCCCTT B	1754
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32516	HCVa:327U21 sense siNA inv stab09	B CCAGAUGCUCUGGAGGGCCTT B	1755
328	CCCGGGAGGUCUCGUAGACCGUG	1418	32517	HCVa:328U21 sense siNA inv stab09	B GCCAGAUGCUCUGGAGGGCTT B	1756
329	CCGGGAGGUCUCGUAGACCGUGC	1419	32518	HCVa:329U21 sense siNA inv stab09	B UGCCAGAUGCUCUGGAGGGTT B	1757
325	UGCCCCGGGAGGUCUCGUAGACC	1394	32519	HCVa:343L21 antisense siNA (325C) inv stab10	GGGGCCCUCCAGAGCAUCUTsT	1758
326	GCCCCGGGAGGUCUCGUAGACCG	1416	32520	HCVa:344L21 antisense siNA (326C) inv stab10	GGGCCCUCCAGAGCAUCUGTsT	1759
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32521	HCVa:345L21 antisense siNA (327C) inv stab10	GGCCCUCCAGAGCAUCUGGTsT	1760
328	CCCGGGAGGUCUCGUAGACCGUG	1418	32522	HCVa:346L21 antisense siNA (328C) inv stab10	GCCCUCCAGAGCAUCUGGCTsT	1761
329	CCGGGAGGUCUCGUAGACCGUGC	1419	32523	HCVa:347L21 antisense siNA (329C) inv stab10	CCCUCCAGAGCAUCUGGCATsT	1762
295	UGUGGUACUGCCUGAUAGGGUGC	1404	32714	HCVa :313L21 antisense siNA (295C) v1 5'p palindrome	pACCCUAUCAGGCAGUACCA GUACUGCCUGAU B	1763
295	UGUGGUACUGCCUGAUAGGGUGC	1404	32715	HCVa :313L21 antisense siNA (295C) v2 5'p palindrome	pACCCUAUCAGGCAGUACC GGUACUGCCUGAU B	1764

327	CCCCGGGAGGUCUCGUAGACCGU	1417	32716	HCVa : 5'p-345L21 antisense (327C) v5 5'p palindrome siNA	pGGUCUACGAGACCUCCCGG AGGUCUCGUAGA B	1765
327	CCCCGGGAGGUCUCGUAGACCGU	1417	32717	HCVa : 5'p-345L21 antisense (327C) v6 5'p palindrome siNA	pGGUCUACGAGACCUCC GGAGGUCUCGUA B	1766
291	GCCUUGUGGUACUGCCUGAUAGG	1403	32796	HCVa:309L21 antisense siNA (291C) stab08 mod pair to #30651	uAucAgGcaguaccAcaAgTsT	1767
295	UGUGGUACUGCCUGAUAGGGUGC	1404	32797	HCVa:313L21 antisense siNA (295C) stab08 mod pair to #30652	acccuaucaggcaguAccaTsT	1768
303	UGCCUGAUAGGGUGCUUGCGAGU	1410	32798	HCVa:321L21 antisense siNA (303C) stab08 mod pair to #30658	ucgcaaGcacccuAucaggTsT	1769
306	CUGAUAGGGUGCUUGCGAGUGCC	1411	32799	HCVa:324L21 antisense siNA (306C) stab08 mod A pair to #30659	cacucgcAagcacccuaucTsT	1770
306	CUGAUAGGGUGCUUGCGAGUGCC	1411	32800	HCVa:324L21 antisense siNA (306C) stab08 mod B pair to #30659	cAcucgcAagcacccuaucTsT	1771
140	UCCCGGGAGAGCCAUAGUGGUCU	1420	33125	HCVa:140U21 sense siNA stab07	B ccGGGAGAGccAuAGuGGuTT B	1772
141	CCCGGGAGAGCCAUAGUGGUCUG	1421	33126	HCVa:141U21 sense siNA stab07	B cGGGAGAGccAuAGuGGucTT B	1773
142	CCGGGAGAGCCAUAGUGGUCUGC	1422	33127	HCVa:142U21 sense siNA stab07	B GGGAGAGccAuAGuGGucuTT B	1774
154	UAGUGGUCUGCGGAACCGGUGAG	1423	33128	HCVa:154U21 sense siNA stab07	B GuGGucuGcGGAAccGGuGTT B	1775
155	AGUGGUCUGCGGAACCGGUGAGU	1424	33129	HCVa:155U21 sense siNA stab07	B uGGucuGcGGAAccGGuGATT B	1776
156	GUGGUCUGCGGAACCGGUGAGUA	1425	33130	HCVa:156U21 sense siNA stab07	B GGucuGcGGAAccGGuGAGTT B	1777
157	UGGUCUGCGGAACCGGUGAGUAC	1426	33131	HCVa:157U21 sense siNA stab07	B GucuGcGGAAccGGuGAGuTT B	1778
158	GGUCUGCGGAACCGGUGAGUACA	1427	33132	HCVa:158U21 sense siNA stab07	B ucuGcGGAAccGGuGAGuATT B	1779
160	UCUGCGGAACCGGUGAGUACACC	1428	33133	HCVa:160U21 sense siNA stab07	B uGcGGAAccGGuGAGuAcATT B	1780
161	CUGCGGAACCGGUGAGUACACCG	1429	33134	HCVa:161U21 sense siNA stab07	B GcGGAAccGGuGAGuAcAcTT B	1781
164	CGGAACCGGUGAGUACACCGGAA	1430	33135	HCVa:164U21 sense siNA stab07	B GAAccGGuGAGuAcAccGGTT B	1782
165	GGAACCGGUGAGUACACCGGAAU	1431	33136	HCVa:165U21 sense siNA stab07	B AAccGGuGAGuAcAccGGATT B	1783
166	GAACCGGUGAGUACACCGGAAUU	1432	33137	HCVa:166U21 sense siNA stab07	B AccGGuGAGuAcAccGGAATT B	1784
167	AACCGGUGAGUACACCGGAAUUG	1433	33138	HCVa:167U21 sense siNA stab07	B ccGGuGAGuAcAccGGAAuTT B	1785
282	UCGCGAAAGGCCUUGUGGUACUG	1434	33139	HCVa:282U21 sense siNA stab07	B GcGAAAGGccuuGuGGuAcTT B	1786
283	CGCGAAAGGCCUUGUGGUACUGC	1435	33140	HCVa:283U21 sense siNA stab07	B cGAAAGGccuuGuGGuAcuTT B	1787
284	GCGAAAGGCCUUGUGGUACUGCC	1436	33141	HCVa:284U21 sense siNA stab07	B GAAAGGccuuGuGGuAcuGTT B	1788
285	CGAAAGGCCUUGUGGUACUGCCU	1437	33142	HCVa:285U21 sense siNA stab07	B AAAGGccuuGuGGuAcuGcTT B	1789
286	GAAAGGCCUUGUGGUACUGCCUG	1438	33143	HCVa:286U21 sense siNA stab07	B AAGGccuuGuGGuAcuGccTT B	1790
288	AAGGCCUUGUGGUACUGCCUGAU	1439	33144	HCVa:288U21 sense siNA stab07	B GGccuuGuGGuAcuGccuGTT B	1791
289	AGGCCUUGUGGUACUGCCUGAUA	1440	33145	HCVa:289U21 sense siNA stab07	B GccuuGuGGuAcuGccuGATT B	1792
290	GGCCUUGUGGUACUGCCUGAUAG	1441	33146	HCVa:290U21 sense siNA stab07	B ccuuGuGGuAcuGccuGAuTT B	1793
299	GUACUGCCUGAUAGGGUGCUUGC	1442	33147	HCVa:299U21 sense siNA stab07	B AcuGccuGAuAGGGuGcuuTT B	1794
302	CUGCCUGAUAGGGUGCUUGCGAG	1443	33148	HCVa:302U21 sense siNA stab07	B GccuGAuAGGGuGcuuGcGTT B	1795

304	GCCUGAUAGGGUGCUUGCGAGUG	1444	33149	HCVa:304U21 sense siNA stab07	B cuGAuAGGGuGcuuGcGAGTT B	1796
305	CCUGAUAGGGUGCUUGCGAGUGC	1445	33150	HCVa:305U21 sense siNA stab07	B uGAuAGGGuGcuuGcGAGuTT B	1797
307	UGAUAGGGUGCUUGCGAGUGCCC	1446	33151	HCVa:307U21 sense siNA stab07	B AuAGGGuGcuuGcGAGuGcTT B	1798
308	GAUAGGGUGCUUGCGAGUGCCCC	1447	33152	HCVa:308U21 sense siNA stab07	B uAGGGuGcuuGcGAGuGccTT B	1799
310	UAGGGUGCUUGCGAGUGCCCCGG	1448	33153	HCVa:310U21 sense siNA stab07	B GGGuGcuuGcGAGuGccccTT B	1800
311	AGGGUGCUUGCGAGUGCCCCGGG	1449	33154	HCVa:311U21 sense siNA stab07	B GGuGcuuGcGAGuGccccGTT B	1801
314	GUGCUUGCGAGUGCCCCGGGAGG	1450	33155	HCVa:314U21 sense siNA stab07	B GcuuGcGAGuGccccGGGATT B	1802
315	UGCUUGCGAGUGCCCCGGGAGGU	1451	33156	HCVa:315U21 sense siNA stab07	B cuuGcGAGuGccccGGGAGTT B	1803
316	GCUUGCGAGUGCCCCGGGAGGUC	1452	33157	HCVa:316U21 sense siNA stab07	B uuGcGAGuGccccGGGAGGTT B	1804
317	CUUGCGAGUGCCCCGGGAGGUCU	1453	33158	HCVa:317U21 sense siNA stab07	B uGcGAGuGccccGGGAGGuTT B	1805
318	UUGCGAGUGCCCCGGGAGGUCUC	1454	33159	HCVa:318U21 sense siNA stab07	B GcGAGuGccccGGGAGGucTT B	1806
319	UGCGAGUGCCCCGGGAGGUCUCG	1455	33160	HCVa:319U21 sense siNA stab07	B cGAGuGccccGGGAGGucuTT B	1807
320	GCGAGUGCCCCGGGAGGUCUCGU	1456	33161	HCVa:320U21 sense siNA stab07	B GAGuGccccGGGAGGucucTT B	1808
322	GAGUGCCCCGGGAGGUCUCGUAG	1457	33162	HCVa:322U21 sense siNA stab07	B GuGccccGGGAGGucucGuTT B	1809
323	AGUGCCCCGGGAGGUCUCGUAGA	1458	33163	HCVa:323U21 sense siNA stab07	B uGccccGGGAGGucucGuATT B	1810
330	CGGGAGGUCUCGUAGACCGUGCA	1459	33164	HCVa:330U21 sense siNA stab07	B GGAGGucucGuAGAccGuGTT B	1811
140	UCCCGGGAGAGCCAUAGUGGUCU	1420	33165	HCVa :158L21 antisense siNA (140C) stab08	AccAcuAuGGcucucccGGTsT	1812
141	CCCGGGAGAGCCAUAGUGGUCUG	1421	33166	HCVa :159L21 antisense siNA (141C) stab08	GaccAcuAuGGcucucccGTsT	1813
142	CCGGGAGAGCCAUAGUGGUCUGC	1422	33167	HCVa :160L21 antisense siNA (142C) stab08	AGAccAcuAuGGcucucccTsT	1814
154	UAGUGGUCUGCGGAACCGGUGAG	1423	33168	HCVa :172L21 antisense siNA (154C) stab08	cAccGGuuccGcAGAccAcTsT	1815
155	AGUGGUCUGCGGAACCGGUGAGU	1424	33169	HCVa :173L21 antisense siNA (155C) stab08	ucAccGGuuccGcAGAccATsT	1816
156	GUGGUCUGCGGAACCGGUGAGUA	1425	33170	HCVa :174L21 antisense siNA (156C) stab08	cucAccGGuuccGcAGAccTsT	1817
157	UGGUCUGCGGAACCGGUGAGUAC	1426	33171	HCVa :175L21 antisense siNA (157C) stab08	AcucAccGGuuccGcAGAcTsT	1818
158	GGUCUGCGGAACCGGUGAGUACA	1427	33172	HCVa :176L21 antisense siNA (158C) stab08	uAcucAccGGuuccGcAGATsT	1819
160	UCUGCGGAACCGGUGAGUACACC	1428	33173	HCVa :178L21 antisense siNA (160C) stab08	uGuAcucAccGGuuccGcATsT	1820
161	CUGCGGAACCGGUGAGUACACCG	1429	33174	HCVa :179L21 antisense siNA (161C) stab08	GuGuAcucAccGGuuccGcTsT	1821
164	CGGAACCGGUGAGUACACCGGAA	1430	33175	HCVa :182L21 antisense siNA (164C) stab08	ccGGuGuAcucAccGGuucTsT	1822
165	GGAACCGGUGAGUACACCGGAAU	1431	33176	HCVa :183L21 antisense siNA (165C) stab08	uccGGuGuAcucAccGGuuTsT	1823

166	GAACCGGUGAGUACACCGGAAUU	1432	33177	HCVa :184L21 antisense siNA (166C) stab08	uuccGGuGuAcucAccGGuTsT	1824
167	AACCGGUGAGUACACCGGAAUUG	1433	33178	HCVa :185L21 antisense siNA (167C) stab08	AuuccGGuGuAcucAccGGTsT	1825
282	UCGCGAAAGGCCUUGUGGUACUG	1434	33179	HCVa :300L21 antisense siNA (282C) stab08	GuAccAcAAGGccuuucGcTsT	1826
283	CGCGAAAGGCCUUGUGGUACUGC	1435	33180	HCVa :301L21 antisense siNA (283C) stab08	AGuAccAcAAGGccuuucGTsT	1827
284	GCGAAAGGCCUUGUGGUACUGCC	1436	33181	HCVa :302L21 antisense siNA (284C) stab08	cAGuAccAcAAGGccuuucTsT	1828
285	CGAAAGGCCUUGUGGUACUGCCU	1437	33182	HCVa :303L21 antisense siNA (285C) stab08	GcAGuAccAcAAGGccuuuTsT	1829
286	GAAAGGCCUUGUGGUACUGCCUG	1438	33183	HCVa :304L21 antisense siNA (286C) stab08	GGcAGuAccAcAAGGccuuTsT	1830
288	AAGGCCUUGUGGUACUGCCUGAU	1439	33184	HCVa :306L21 antisense siNA (288C) stab08	cAGGcAGuAccAcAAGGccTsT	1831
289	AGGCCUUGUGGUACUGCCUGAUA	1440	33185	HCVa :307L21 antisense siNA (289C) stab08	ucAGGcAGuAccAcAAGGcTsT	1832
290	GGCCUUGUGGUACUGCCUGAUAG	1441	33186	HCVa :308L21 antisense siNA (290C) stab08	AucAGGcAGuAccAcAAGGTsT	1833
299	GUACUGCCUGAUAGGGUGCUUGC	1442	33187	HCVa :317L21 antisense siNA (299C) stab08	AAGcAcccuAucAGGcAGuTsT	1834
302	CUGCCUGAUAGGGUGCUUGCGAG	1443	33188	HCVa :320L21 antisense siNA (302C) stab08	cGcAAGcAcccuAucAGGcTsT	1835
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	33189	HCVa :322L21 antisense siNA (304C) stab08	cucGcAAGcAcccuAucAGTsT	1836
305	CCUGAUAGGGUGCUUGCGAGUGC	1445	33190	HCVa :323L21 antisense siNA (305C) stab08	AcucGcAAGcAcccuAucATsT	1837
307	UGAUAGGGUGCUUGCGAGUGCCC	1446	33191	HCVa :325L21 antisense siNA (307C) stab08	GcAcucGcAAGcAcccuAuTsT	1838
308	GAUAGGGUGCUUGCGAGUGCCCC	1447	33192	HCVa :326L21 antisense siNA (308C) stab08	GGcAcucGcAAGcAcccuATsT	1839
310	UAGGGUGCUUGCGAGUGCCCCGG	1448	33193	HCVa :328L21 antisense siNA (310C) stab08	GGGGcAcucGcAAGcAcccTsT	1840
311	AGGGUGCUUGCGAGUGCCCCGGG	1449	33194	HCVa :329L21 antisense siNA (311C) stab08	cGGGGcAcucGcAAGcAccTsT	1841
314	GUGCUUGCGAGUGCCCCGGGAGG	1450	33195	HCVa :332L21 antisense siNA (314C) stab08	ucccGGGGcAcucGcAAGcTsT	1842
315	UGCUUGCGAGUGCCCCGGGAGGU	1451	33196	HCVa :333L21 antisense siNA (315C) stab08	cucccGGGGcAcucGcAAGTsT	1843
316	GCUUGCGAGUGCCCCGGGAGGUC	1452	33197	HCVa :334L21 antisense siNA (316C) stab08	ccucccGGGGcAcucGcAATsT	1844
317	CUUGCGAGUGCCCCGGGAGGUCU	1453	33198	HCVa :335L21 antisense siNA	AccucccGGGGcAcucGcATsT	1845

				(317C) stab08		
318	UUGCGAGUGCCCCGGGAGGUCUC	1454	33199	HCVa :336L21 antisense siNA (318C) stab08	GAccucccGGGGcAcucGcTsT	1846
319	UGCGAGUGCCCCGGGAGGUCUCG	1455	33200	HCVa :337L21 antisense siNA (319C) stab08	AGAccucccGGGGcAcucGTsT	1847
320	GCGAGUGCCCCGGGAGGUCUCGU	1456	33201	HCVa :338L21 antisense siNA (320C) stab08	GAGAccucccGGGGcAcucTsT	1848
322	GAGUGCCCCGGGAGGUCUCGUAG	1457	33202	HCVa :340L21 antisense siNA (322C) stab08	AcGAGAccucccGGGGcAcTsT	1849
323	AGUGCCCCGGGAGGUCUCGUAGA	1458	33203	HCVa :341L21 antisense siNA (323C) stab08	uAcGAGAccucccGGGGcATsT	1850
330	CGGGAGGUCUCGUAGACCGUGCA	1459	33204	HCVa :348L21 antisense siNA (330C) stab08	cAcGGucuAcGAGAccuccTsT	1851
303	UGCCUGAUAGGGUGCUUGCGAGU	1410	33329	HCVa :321L21 antisense siNA (303C) stab08 + 5' P	pucGcAAGcAcccuAucAGGTsT	1852
303	UGCCUGAUAGGGUGCUUGCGAGU	1410	33330	HCVa :321L21 antisense siNA (303C) stab05 + 5' P	pucGcAAGcAcccuAucAGGTsT	1853
295	UGUGGUACUGCCUGAUAGGGUGC	1404	33331	HCVa :313L21 antisense siNA (295C) stab05 + 5' P	pAcccuAucAGGcAGuAccATsT	1854
295	UGUGGUACUGCCUGAUAGGGUGC	1404	33332	HCVa :313L21 antisense siNA (295C) stab08 + 5' P	pAcccuAucAGGcAGuAccATsT	1855
306	CUGAUAGGGUGCUUGCGAGUGCC	1411	33333	HCVa :324L21 antisense siNA (306C) stab08 + 5' P	pcAcucGcAAGcAcccuAucTsT	1856
327	CCCCGGGAGGUCUCGUAGACCGU	1417	33334	HCVa :345L21 antisense siNA (327C) stab08 + 5' P	pGGucuAcGAGAccucccGGTsT	1857
303	UGCCUGAUAGGGUGCUUGCGAGU	1410	33346	HCVa :321L21 antisense siNA (303C) stab08 + 5' aminoL	L ucGcAAGcAcccuAucAGGTsT	1858
303	UGCCUGAUAGGGUGCUUGCGAGU	1410	33347	HCVa :321L21 antisense siNA (303C) stab05 + 5' aminoL	L ucGcAAGcAcccuAucAGGTsT	1859
295	UGUGGUACUGCCUGAUAGGGUGC	1404	33348	HCVa :313L21 antisense siNA (295C) stab05 + 5' aminoL	L AcccuAucAGGcAGuAccATsT	1860
295	UGUGGUACUGCCUGAUAGGGUGC	1404	33349	HCVa :313L21 antisense siNA (295C) stab08 + 5' aminoL	L AcccuAucAGGcAGuAccATsT	1861
306	CUGAUAGGGUGCUUGCGAGUGCC	1411	33350	HCVa :324L21 antisense siNA (306C) stab08 + 5' aminoL	L cAcucGcAAGcAcccuAucTsT	1862
327	CCCCGGGAGGUCUCGUAGACCGU	1417	33351	HCVa :345L21 antisense siNA (327C) stab08 + 5' aminoL	L GGucuAcGAGAccucccGGTsT	1863
327	CCCCGGGAGGUCUCGUAGACCGU	1417	34024	HCVa:327U21 sense siNA inact1 stab07	B ccGAGAGGucGcGuAGuccTT B	1864
327	CCCCGGGAGGUCUCGUAGACCGU	1417	34025	HCVa:327U21 sense siNA inact2 stab07	B ccGAGAGGucGcGucGAucTT B	1865
327	CCCCGGGAGGUCUCGUAGACCGU	1417	34026	HCVa:327U21 sense siNA inact3 stab07	B ccGGuAGGucccGuGGAcATT B	1866

327	CCCCGGGAGGUCUCGUAGACCGU	1417	34027	HCVa :345L21 antisense siNA (327C) inact1 stab08	GGAcuAcGcGAccucucGGTsT	1867
327	CCCCGGGAGGUCUCGUAGACCGU	1417	34028	HCVa :345L21 antisense siNA (327C) inact2 stab08	GAucGAcGcGAccucucGGTsT	1868
327	CCCCGGGAGGUCUCGUAGACCGU	1417	34029	HCVa :345L21 antisense siNA (327C) inact3 stab08	uGuccAcGGGAccuAccGGTsT	1869
282	UCGCGAAAGGCCUUGUGGUACUG	1434	34030	HCVa:282U21 sense siNA inact1 stab07	B GcuAAAGGcGuuGuGGcAcTT B	1870
282	UCGCGAAAGGCCUUGUGGUACUG	1434	34031	HCVa:282U21 sense siNA inact2 stab07	B GcGuAAGGcccuGuGGuAATT B	1871
282	UCGCGAAAGGCCUUGUGGUACUG	1434	34032	HCVa:282U21 sense siNA inact3 stab07	B GAGAAAcGccuGGuGGuucTT B	1872
283	CGCGAAAGGCCUUGUGGUACUGC	1435	34033	HCVa:283U21 sense siNA inact1 stab07	B cGuAAGGcAuuGuGGcAcuTT B	1873
283	CGCGAAAGGCCUUGUGGUACUGC	1435	34034	HCVa:283U21 sense siNA inact2 stab07	B cGAGAGGcAuuGuGcuAcuTT B	1874
283	CGCGAAAGGCCUUGUGGUACUGC	1435	34035	HCVa:283U21 sense siNA inact3 stab07	B ccAAAGGucuuGAGGuGcuTT B	1875
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	34036	HCVa:304U21 sense siNA inact1 stab07	B cGGAuAGGcuGcuuGuGAGTT B	1876
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	34037	HCVa:304U21 sense siNA inact2 stab07	B cuGcuAGGGuAcuuGGGAGTT B	1877
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	34038	HCVa:304U21 sense siNA inact3 stab07	B ccGAuAuGGuGAuuGcGGGTT B	1878
307	UGAUAGGGUGCUUGCGAGUGCCC	1446	34039	HCVa:307U21 sense siNA inact1 stab07	B AuuGGGuGcuGGcGAGuAcTT B	1879
307	UGAUAGGGUGCUUGCGAGUGCCC	1446	34040	HCVa:307U21 sense siNA inact2 stab07	B AuAuGGuGccuGcGAGuGGTT B	1880
307	UGAUAGGGUGCUUGCGAGUGCCC	1446	34041	HCVa:307U21 sense siNA inact3 stab07	B AGAGGGuAcuuGcGcGuGuTT B	1881
282	UCGCGAAAGGCCUUGUGGUACUG	1434	34042	HCVa :300L21 antisense siNA (282C) inact1 stab08	GuGccAcAAcGccuuuAGcTsT	1882
282	UCGCGAAAGGCCUUGUGGUACUG	1434	34043	HCVa :300L21 antisense siNA (282C) inact2 stab08	uuAccAcAGGGccuuAcGcTsT	1883
282	UCGCGAAAGGCCUUGUGGUACUG	1434	34044	HCVa :300L21 antisense siNA (282C) inact3 stab08	GAAccAccAGGcGuuucucTsT	1884
283	CGCGAAAGGCCUUGUGGUACUGC	1435	34045	HCVa :301L21 antisense siNA (283C) inact1 stab08	AguGccAcAAuGccuuAcGTsT	1885
283	CGCGAAAGGCCUUGUGGUACUGC	1435	34046	HCVa :301L21 antisense siNA (283C) inact2 stab08	AguAGcAcAAuGccucucGTsT	1886
283	CGCGAAAGGCCUUGUGGUACUGC	1435	34047	HCVa :301L21 antisense siNA (283C) inact3 stab08	AGcAccucAAGAccuuuGGTsT	1887
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	34048	HCVa :322L21 antisense siNA	cucAcAAGcAGccuAuccGTsT	1888

				(304C) inact1 stab08		
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	34049	HCVa :322L21 antisense siNA (304C) inact2 stab08	cucccAAGuAcccuAGcAGTsT	1889
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	34050	HCVa :322L21 antisense siNA (304C) inact3 stab08	cccGcAAucAccAuAucGGTsT	1890
307	UGAUAGGGUGCUUGCGAGUGCCC	1446	34051	HCVa :325L21 antisense siNA (307C) inact1 stab08	GuAcucGccAGcAcccAAuTsT	1891
307	UGAUAGGGUGCUUGCGAGUGCCC	1446	34052	HCVa :325L21 antisense siNA (307C) inact2 stab08	ccAcucGcAGGcAccAuAuTsT	1892
307	UGAUAGGGUGCUUGCGAGUGCCC	1446	34053	HCVa :325L21 antisense siNA (307C) inact3 stab08	AcAcGcGcAAGuAcccucuTsT	1893
282	UCGCGAAAGGCCUUGUGGUACUG	1434	34054	HCVa:282U21 sense siNA inv stab07	B cAuGGuGuuccGGAAAGcGTT B	1894
283	CGCGAAAGGCCUUGUGGUACUGC	1435	34055	HCVa:283U21 sense siNA inv stab07	B ucAuGGuGuuccGGAAAGcTT B	1895
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	34056	HCVa:304U21 sense siNA inv stab07	B GAGcGuucGuGGGAuAGucTT B	1896
307	UGAUAGGGUGCUUGCGAGUGCCC	1446	34057	HCVa:307U21 sense siNA inv stab07	B cGuGAGcGuucGuGGGAuATT B	1897
282	UCGCGAAAGGCCUUGUGGUACUG	1434	34058	HCVa:300L21 antisense siNA (282C) inv stab08	cGcuuuccGGAAcAccAuGTsT	1898
283	CGCGAAAGGCCUUGUGGUACUGC	1435	34059	HCVa:301L21 antisense siNA (283C) inv stab08	GcuuuccGGAAcAccAuGATsT	1899
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	34060	HCVa:322L21 antisense siNA (304C) inv stab08	GAcuAucccAcGAAcGcucTsT	1900
307	UGAUAGGGUGCUUGCGAGUGCCC	1446	34061	HCVa:325L21 antisense siNA (307C) inv stab08	uAucccAcGAAcGcucAcGTsT	1901
82	UAGCCAUGGCGUUAGUAUGAGUG	1460	34128	HCVb :100L18 (82C) 5'p palindrome siNA	pUCAUACUAACGCCAUGGC GUUAGUAUGAB	1902
82	UAGCCAUGGCGUUAGUAUGAGUG	1460	34129	HCVb :100L17 (82C) 5'p palindrome siNA	pCAUACUAACGCCAUGGC GUUAGUAUGB	1903
82	UAGCCAUGGCGUUAGUAUGAGUG	1460	34130	HCVb :100L16 (82C) 5'p palindrome siNA	pAUACUAACGCCAUGGC GUUAGUAUB	1904
82	UAGCCAUGGCGUUAGUAUGAGUG	1460	34131	HCVb :100L15 (82C) 5'p palindrome siNA	pUACUAACGCCAUGGC GUUAGUAB	1905
126	CCCUCCCGGGAGAGCCAUAGUGG	1461	34132	HCVb :144L19 (126C) 5'p palindrome siNA	pACUAUGGCUCUCCCGGGAG AGCCAUAGUB	1906
126	CCCUCCCGGGAGAGCCAUAGUGG	1461	34133	HCVb :144L18 (126C) 5'p palindrome siNA	pCUAUGGCUCUCCCGGGAG AGCCAUAGB	1907
126	CCCUCCCGGGAGAGCCAUAGUGG	1461	34134	HCVb :144L17 (126C) 5'p palindrome siNA	pUAUGGCUCUCCCGGGAG AGCCAUAB	1908
126	CCCUCCCGGGAGAGCCAUAGUGG	1461	34135	HCVb :144L16 (126C) 5'p palindrome siNA	pAUGGCUCUCCCGGGAG AGCCAUB	1909
126	CCCUCCCGGGAGAGCCAUAGUGG	1461	34136	HCVb :144L15 (126C) 5'p palindrome siNA	pUGGCUCUCCCGGGAG AGCCAB	1910
155	GAACCGGUGAGUACACCGGAAUU	1432	34137	HCVb :171L17 (155C) 5'p	pCCGGUGUACUCACCGGU GAGUACACCGGB	1911

				palindrome siNA		
155	GAACCGGUGAGUACACCGGAAUU	1432	34138	HCVb :170L16 (155C) 5'p palindrome siNA	pCGGUGUACUCACCGGU GAGUACACCGB	1912
155	GAACCGGUGAGUACACCGGAAUU	1432	34139	HCVb :169L15 (155C) 5'p palindrome siNA	pGGUGUACUCACCGGU GAGUACACCB	1913
315	GCCCCGGGAGGUCUCGUAGACCG	1416	34140	HCVb :331L17 (315C) 5'p palindrome siNA	pCUACGAGACCUCCCGGG AGGUCUCGUAGB	1914
315	GCCCCGGGAGGUCUCGUAGACCG	1416	34141	HCVb :330L16 (315C) 5'p palindrome siNA	pUACGAGACCUCCCGGG AGGUCUCGUAB	1915
315	GCCCCGGGAGGUCUCGUAGACCG	1416	34142	HCVb :329L15 (315C) 5'p palindrome siNA	pACGAGACCUCCCGGG AGGUCUCGUB	1916
327	CCCCGGGAGGUCUCGUAGACCGU	1417	34494	HCVa :345L21 antisense siNA (327C) stab19	GGucuAcGAGAccucccGGTT B	1917
327	CCCCGGGAGGUCUCGUAGACCGU	1417	34495	HCVa:345L21 antisense siNA (327C) inv stab19	GGcccuccAGAGcAucuGGTT B	1918
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	34496	HCVa :322L21 antisense siNA (304C) stab19	cucGcAAGcAcccuAucAGTT B	1919
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	34499	HCVa:322L21 antisense siNA (304C) inv stab19	GAcuAucccAcGAAcGcucTT B	1920
282	UCGCGAAAGGCCUUGUGGUACUG	1434	34581	HCVa:282U21 sense siNA stab00	GCGAAAGGCCUUGUGGUACTT	1921
283	CGCGAAAGGCCUUGUGGUACUGC	1435	34582	HCVa:283U21 sense siNA stab00	CGAAAGGCCUUGUGGUACUTT	1922
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	34583	HCVa:304U21 sense siNA stab00	CUGAUAGGGUGCUUGCGAGTT	1923
307	UGAUAGGGUGCUUGCGAGUGCCC	1446	34584	HCVa:307U21 sense siNA stab00	AUAGGGUGCUUGCGAGUGCTT	1924
327	CCCCGGGAGGUCUCGUAGACCGU	1417	34585	HCVa:327U21 sense siNA stab00	CCGGGAGGUCUCGUAGACCTT	1925
282	UCGCGAAAGGCCUUGUGGUACUG	1434	34586	HCVa :300L21 antisense siNA (282C) stab00	GUACCACAAGGCCUUUCGCTT	1926
283	CGCGAAAGGCCUUGUGGUACUGC	1435	34587	HCVa :301L21 antisense siNA (283C) stab00	AGUACCACAAGGCCUUUCGTT	1927
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	34588	HCVa :322L21 antisense siNA (304C) stab00	CUCGCAAGCACCCUAUCAGTT	1928
307	UGAUAGGGUGCUUGCGAGUGCCC	1446	34589	HCVa :325L21 antisense siNA (307C) stab00	GCACUCGCAAGCACCCUAUTT	1929
282	UCGCGAAAGGCCUUGUGGUACUG	1434	34590	HCVa:282U21 sense siNA inv stab00	CAUGGUGUUCCGGAAAGCGTT	1930
283	CGCGAAAGGCCUUGUGGUACUGC	1435	34591	HCVa:283U21 sense siNA inv stab00	UCAUGGUGUUCCGGAAAGCTT	1931
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	34592	HCVa:304U21 sense siNA inv stab00	GAGCGUUCGUGGGAUAGUCTT	1932
307	UGAUAGGGUGCUUGCGAGUGCCC	1446	34593	HCVa:307U21 sense siNA inv stab00	CGUGAGCGUUCGUGGGAUATT	1933
327	CCCCGGGAGGUCUCGUAGACCGU	1417	34594	HCVa:327U21 sense siNA inv stab00	CCAGAUGCUCUGGAGGGCCTT	1934
282	UCGCGAAAGGCCUUGUGGUACUG	1434	34595	HCVa:300L21 antisense siNA (282C) inv stab00	CGCUUUCCGGAACACCAUGTT	1935
283	CGCGAAAGGCCUUGUGGUACUGC	1435	34596	HCVa:301L21 antisense siNA (283C) inv stab00	GCUUUCCGGAACACCAUGATT	1936

304	GCCUGAUAGGGUGCUUGCGAGUG	1444	34597	HCVa:322L21 antisense siNA (304C) inv stab00	GACUAUCCCACGAACGCUCTT	1937
307	UGAUAGGGUGCUUGCGAGUGCCC	1446	34598	HCVa:325L21 antisense siNA (307C) inv stab00	UAUCCCACGAACGCUCACGTT	1938
327	CCCCGGGAGGUCUCGUAGACCGU	1417	34599	HCVa:345L21 antisense siNA (327C) inv stab00	GGCCCUCCAGAGCAUCUGGTT	1939
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35173	HCVa:327U21 sense siNA stab07 N1	B ccGGGAGGucucGUAGACCTT B	1940
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35174	HCVa :345L21 antisense siNA (327C) stab08 N1	GGUCUAcGAGAccucccGGTsT	1941
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35175	HCVa :345L21 antisense siNA (327C) stab25	GGUcuAcGAGAccucccGGTsT	1942
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35176	HCVa :345L21 antisense siNA (327C) stab08 N3	GGucuAcGAGAccucccGGTsT	1943
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35177	HCVa :345L21 antisense siNA (327C) stab24	GGucuAcGAGAccucccGGTsT	1944
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	35178	HCVa:304U21 sense siNA stab07 N1	B cuGAuAGGGuGcuUGCGAGTT B	1945
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	35179	HCVa :322L21 antisense siNA (304C) stab08 N1	CUCGCAAGcAcccuAucAGTsT	1946
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	35180	HCVa :322L21 antisense siNA (304C) stab25	CUCGcAAGcAcccuAucAGTsT	1947
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	35181	HCVa :322L21 antisense siNA (304C) stab08 N3	CUcGcAAGcAcccuAucAGTsT	1948
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	35182	HCVa :322L21 antisense siNA (304C) stab24	CucGcAAGcAcccuAucAGTsT	1949
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35225	HCVa:327 siNA stab0/0 Pal01	GGUCUACGAGACCUCCCGG CCGGGAGGUCUCGUAGACC	1950
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35226	HCVa:327 siNA stab0/0 Pal02	GGUCUACGAGACCUCCCGG CCGGGAGGUCUCGUAGACCTT	1951
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35227	HCVa:327 siNA stab0/0 Pal03	GGUCUACGAGACCUCCCG CGGGAGGUCUCGUAGACC	1952
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35228	HCVa:327 siNA stab0/0 Pal04	GGUCUACGAGACCUCCCG CGGGAGGUCUCGUAGACCTT	1953
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35229	HCVa:327 siNA stab0/0 Pal05	GGUCUACGAGACCUCCC GGGAGGUCUCGUAGACC	1954
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35230	HCVa:327 siNA stab0/0 Pal06	GGUCUACGAGACCUCCC GGGAGGUCUCGUAGACCTT	1955
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35231	HCVa:327 siNA stab0/0 Pal07	GGUCUACGAGACCUCC GGAGGUCUCGUAGACC	1956
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35232	HCVa:327 siNA stab0/0 Pal08	GGUCUACGAGACCUCC GGAGGUCUCGUAGACCTT	1957
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35235	HCVa:327 siNA stab0/0 Pal11	GUCUACGAGACCUCCCGG GAGGUCUCGUAGAC	1958

327	CCCCGGGAGGUCUCGUAGACCGU	1417	35236	HCVa:327 siNA stab0/0 Pal12	GUCUACGAGACCUCCCGG GAGGUCUCGUAGACTT	1959
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35237	HCVa:327 siNA stab0/0 Pal13	UCUACGAGACCUCCCGG GAGGUCUCGUAGA	1960
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35238	HCVa:327 siNA stab0/0 Pal14	UCUACGAGACCUCCCGG GAGGUCUCGUAGATT	1961
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35239	HCVa:327 siNA stab0/0 Pal15	CUACGAGACCUCCCGG GAGGUCUCGUAG	1962
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35240	HCVa:327 siNA stab0/0 Pal16	CUACGAGACCUCCCGG GAGGUCUCGUAGTT	1963
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35241	HCVa:327 siNA stab0/0 Pal17	GGUCUACGAGACCUCCAGG UCUCGUAGACC	1964
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35242	HCVa:327 siNA stab0/0 Pal18	GGUCUACGAGACCUCCAGG UCUCGUAGACCTT	1965
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35243	HCVa:327 siNA stab0/0 Pal19	GGUCUACGAGACCUCGAGG UCUCGUAGACC	1966
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35244	HCVa:327 siNA stab0/0 Pal20	GGUCUACGAGACCUCGAGG UCUCGUAGACCTT	1967
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35245	HCVa:327 siNA stab0/0 Pal21	GGUCUACGAGACCUGCAGG UCUCGUAGACC	1968
327	CCCCGGGAGGUCUCGUAGACCGU	1417	35246	HCVa:327 siNA stab0/0 Pal22	GGUCUACGAGACCUGCAGG UCUCGUAGACCTT	1969
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	35247	HCVa:304 siNA stab0/0 Pal01	GACUAUCCCACGAACGCUC GAGCGUUCGUGGGAUAGUCTT	1970
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	35248	HCVa:304 siNA stab0/0 Pal02	GACUAUCCCACGAACGCUC GAGCGUUCGUGGGAUAGUC	1971
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	35249	HCVa:304 siNA stab0/0 Pal03	GACUAUCCCACGAACGCGU UCGUGGGAUAGUCTT	1972
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	35250	HCVa:304 siNA stab0/0 Pal04	GACUAUCCCACGAACGCGU UCGUGGGAUAGUC	1973
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	35251	HCVa:304 siNA stab0/0 Pal05	GACUAUCCCACGAACGUUC GUGGGAUAGUCTT	1974
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	35252	HCVa:304 siNA stab0/0 Pal06	GACUAUCCCACGAACGUUC GUGGGAUAGUC	1975
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	35253	HCVa:304 siNA stab0/0 Pal07	ACUAUCCCACGAACGUUC GUGGGAUAGUTT	1976
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	35254	HCVa:304 siNA stab0/0 Pal08	ACUAUCCCACGAACGUUC GUGGGA	1977
327	CCCCGGGAGGUCUCGUAGACCGU UCGCGAAAGGCCUUGUGGUACUG	1462	36414	HCVa bf-L-21 siNA stab00 [HCVa:327U21 sense o18S HCVa:282U21 sense]	CCGGGAGGUCUCGUAGACCTT L GCGAAAGGCCUUGUGGUACTT	1978
327	CCCCGGGAGGUCUCGUAGACCGU UGAUAGGGUGCUUGCGAGUGCCC	1463	36415	HCVa bf-L-22 siNA stab00 [HCVa:327U21 sense o18S HCVa:307U21 sense]	CCGGGAGGUCUCGUAGACCTT L AUAGGGUGCUUGCGAGUGCTT	1979
307	UGAUAGGGUGCUUGCGAGUGCCC UCGCGAAAGGCCUUGUGGUACUG	1464	36430	HCVa bf-L-20 siNA stab00 [HCVa:307U21 sense o18S HCVa:282U21 sense]	AUAGGGUGCUUGCGAGUGCTT L GCGAAAGGCCUUGUGGUACTT	1980
307	UGAUAGGGUGCUUGCGAGUGCCC	1446	36438	HCVa:307U21 sense siNA stab00	AUAGGGUGCUUGCGAGUGCTT	1924
307	UGAUAGGGUGCUUGCGAGUGCCC	1446	36446	HCVa :325L21 antisense siNA (307C) stab00	GCACUCGCAAGCACCCUAUTT	1929

327	CCCCGGGAGGUCUCGUAGACCGU	1417	36447	HCVa :345L21 antisense siNA (327C) stab00	GGUCUACGAGACCUCCCGGTT	1732
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	36727	HCVa:304U21 sense siNA stab09	B CUGAUAGGGUGCUUGCGAGTT B	1981
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	36728	HCVa :322L21 antisense siNA (304C) stab10	CUCGCAAGCACCCUAUCAGTsT	1982
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	37010	HCVa:304U21 sense siNA stab04	B cuGAuAGGGuGcuuGcGAGTT B	1983
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	37011	HCVa :322L21 antisense siNA (304C) stab05	cucGcAAGcAcccuAucAGTsT	1984
307	CCCCGGGAGGUCUCGUAGACCGU UGAUAGGGUGCUUGCGAGUGCCC	1463	37781	HCVa bf-L-22 siNA stab07 [HCVa:327U21 sense o18S HCVa:307U21 sense]	B ccGGGAGGucucGuAGAccTT L AuAGGGuGcuuGcGAGuGcTT B	1985
307	UGAUAGGGUGCUUGCGAGUGCCC	1446	37790	HCVa :325L21 antisense siNA (307C) stab26	GCAcucGcAAGcAcccuAuTT	1986
327	CCCCGGGAGGUCUCGUAGACCGU	1417	37791	HCVa :345L21 antisense siNA (327C) stab26	GGUcuAcGAGAccucccGGTT	1987
282	UCGCGAAAGGCCUUGUGGUACUG	1434	38279	HCVa :300L21 antisense siNA (282C) stab25	GUAccAcAAGGccuuucGcTsT	1988
283	CGCGAAAGGCCUUGUGGUACUGC	1435	38280	HCVa :301L21 antisense siNA (283C) stab25	AGUAccAcAAGGccuuucGTsT	1989
307	UGAUAGGGUGCUUGCGAGUGCCC	1446	38281	HCVa :325L21 antisense siNA (307C) stab25	GCAcucGcAAGcAcccuAuTsT	1990
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	38283	HCVa :322L21 antisense siNA (304C) stab26	CUCGcAAGcAcccuAucAGTT	1991
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	38284	HCVa :322L21 antisense siNA (304C) stab27	CUCGcAAGcAcccuAucAGTTB	1992
282	UCGCGAAAGGCCUUGUGGUACUG	1434	38293	HCVa :300L21 antisense siNA (282C) stab19	GuAccAcAAGGccuuucGcTT B	1993
282	UCGCGAAAGGCCUUGUGGUACUG	1434	38294	HCVa :300L21 antisense siNA (282C) stab26	GUAccAcAAGGccuuucGcTT	1994
282	UCGCGAAAGGCCUUGUGGUACUG	1434	38295	HCVa :300L21 antisense siNA (282C) stab27	GUAccAcAAGGccuuucGcTT B	1995
282	UCGCGAAAGGCCUUGUGGUACUG	1434	38296	HCVa :300L21 antisense siNA (282C) stab29	GuAccAcAAGGccuuucGcTsT	1996
282	UCGCGAAAGGCCUUGUGGUACUG	1434	38297	HCVa :300L21 antisense siNA (282C) stab30	GuAccAcAAGGccuuucGcTT	1997
282	UCGCGAAAGGCCUUGUGGUACUG	1434	38298	HCVa :300L21 antisense siNA (282C) stab31	GuAccAcAAGGccuuucGcTT B	1998
282	UCGCGAAAGGCCUUGUGGUACUG	1434	38299	HCVa :300L21 antisense siNA (282C) stab32	GuAccAcAAGGccuuucGcTT	1999
304	GCCUGAUAGGGUGCUUGCGAGUG	1444	38300	HCVa :322L21 antisense siNA (304C) stab32	cucGcAAGcAcccuAucAGTT	2000
327	CCCCGGGAGGUCUCGUAGACCGU	1417	38301	HCVa :345L21 antisense siNA	GGUcuAcGAGAccucccGGTT B	2001

				(327C) stab27		
327	CCCCGGGAGGUCUCGUAGACCGU	1417	38302	HCVa :345L21 antisense siNA (327C) stab30	GGucuAcGAGAccucccGGTT	2002
327	CCCCGGGAGGUCUCGUAGACCGU	1417	38303	HCVa :345L21 antisense siNA (327C) stab31	GGucuAcGAGAccucccGGTT B	2003
327	CCCCGGGAGGUCUCGUAGACCGU	1417	38304	HCVa :345L21 antisense siNA (327C) stab32	GGucuAcGAGAccucccGGTT	2004
304	CCCCGGGAGGUCUCGUAGACCGU GCCUGAUAGGGUGCUUGCGAGUG	1465	38310	HCV bf-L-23 siNA stab00 [HCV:327U21 sense o18S HCV:304U21 sense]	CCGGGAGGUCUCGUAGACCTT L CUGAUAGGGUGCUUGCGAGTT	2005
282	GCCUGAUAGGGUGCUUGCGAGUG UCGCGAAAGGCCUUGUGGUACUG	1466	38311	HCV bf-L-24 siNA stab00 [HCV:304U21 sense o18S HCV:282U21 sense]	CUGAUAGGGUGCUUGCGAGTT L GCGAAAGGCCUUGUGGUACTT	2006
304	CCCCGGGAGGUCUCGUAGACCGU GCCUGAUAGGGUGCUUGCGAGUG	1465	38312	HCV bf-L-23 siNA stab07 [HCV:327U21 sense o18S HCV:304U21 sense]	B ccGGGAGGucucGuAGAccTT L cuGAuAGGGuGcuuGcGAGTT B	2007
282	CCCCGGGAGGUCUCGUAGACCGU UCGCGAAAGGCCUUGUGGUACUG	1462	38313	HCV bf-L-21 siNA stab07 [HCVa:327U21 sense o18S HCVa:282U21 sense]	B ccGGGAGGucucGuAGAccTT L GcGAAAGGccuuGuGGuAcTT B	2008
282	GCCUGAUAGGGUGCUUGCGAGUG UCGCGAAAGGCCUUGUGGUACUG	1466	38314	HCV bf-L-24 siNA stab07 [HCV:304U21 sense o18S HCV:282U21 sense]	B cuGAuAGGGuGcuuGcGAGTT L GcGAAAGGccuuGuGGuAcTT B	2009

Uppercase = ribonucleotide
u,c = 2'-deoxy-2'-fluoro U,C
T = thymidine
B = inverted deoxy abasic
s = phosphorothioate linkage
A = deoxy Adenosine
G = deoxy Guanosine
G = 2'-O-methyl Guanosine
A = 2'-O-methyl Adenosine
L = hegS = hexethelyne glycol spacer; spacer-18 (Glen Research 10-1918-xx)
p = terminal phosphate

Table IV

Non-limiting examples of Stabilization Chemistries for chemically modified siNA constructs

Chemistry	pyrimidine	Purine	cap	p=S	Strand
"Stab 00"	Ribo	Ribo	TT at 3'-ends		S/AS
"Stab 1"	Ribo	Ribo	-	5 at 5'-end 1 at 3'-end	S/AS
"Stab 2"	Ribo	Ribo	-	All linkages	Usually AS
"Stab 3"	2'-fluoro	Ribo	-	4 at 5'-end 4 at 3'-end	Usually S
"Stab 4"	2'-fluoro	Ribo	5' and 3'-ends	-	Usually S
"Stab 5"	2'-fluoro	Ribo	-	1 at 3'-end	Usually AS
"Stab 6"	2'-O-Methyl	Ribo	5' and 3'-ends	-	Usually S
"Stab 7"	2'-fluoro	2'-deoxy	5' and 3'-ends	-	Usually S
"Stab 8"	2'-fluoro	2'-O-Methyl	-	1 at 3'-end	S/AS
"Stab 9"	Ribo	Ribo	5' and 3'-ends	-	Usually S
"Stab 10"	Ribo	Ribo	-	1 at 3'-end	Usually AS
"Stab 11"	2'-fluoro	2'-deoxy	-	1 at 3'-end	Usually AS
"Stab 12"	2'-fluoro	LNA	5' and 3'-ends		Usually S
"Stab 13"	2'-fluoro	LNA		1 at 3'-end	Usually AS
"Stab 14"	2'-fluoro	2'-deoxy		2 at 5'-end 1 at 3'-end	Usually AS
"Stab 15"	2'-deoxy	2'-deoxy		2 at 5'-end 1 at 3'-end	Usually AS
"Stab 16"	Ribo	2'-O-Methyl	5' and 3'-ends		Usually S
"Stab 17"	2'-O-Methyl	2'-O-Methyl	5' and 3'-ends		Usually S
"Stab 18"	2'-fluoro	2'-O-Methyl	5' and 3'-ends		Usually S
"Stab 19"	2'-fluoro	2'-O-Methyl	3'-end		S/AS
"Stab 20"	2'-fluoro	2'-deoxy	3'-end		Usually AS
"Stab 21"	2'-fluoro	Ribo	3'-end		Usually AS
"Stab 22"	Ribo	Ribo	3'-end		Usually AS
"Stab 23"	2'-fluoro*	2'-deoxy*	5' and 3'-ends		Usually S
"Stab 24"	2'-fluoro*	2'-O-Methyl*	-	1 at 3'-end	S/AS
"Stab 25"	2'-fluoro*	2'-O-Methyl*	-	1 at 3'-end	S/AS

"Stab 26"	2'-fluoro*	2'-O-Methyl*	-		S/AS
"Stab 27"	2'-fluoro*	2'-O-Methyl*	3'-end		S/AS
"Stab 28"	2'-fluoro*	2'-O-Methyl*	3'-end		S/AS
"Stab 29"	2'-fluoro*	2'-O-Methyl*		1 at 3'-end	S/AS
"Stab 30"	2'-fluoro*	2'-O-Methyl*			S/AS
"Stab 31"	2'-fluoro*	2'-O-Methyl*	3'-end		S/AS
"Stab 32"	2'-fluoro	2'-O-Methyl			S/AS

CAP = any terminal cap, see for example **Figure 10**.
All Stab 00-32 chemistries can comprise 3'-terminal thymidine (TT) residues
All Stab 00-32 chemistries typically comprise about 21 nucleotides, but can vary as described herein.
S = sense strand
AS = antisense strand
*Stab 23 has a single ribonucleotide adjacent to 3'-CAP
*Stab 24 and Stab 28 have a single ribonucleotide at 5'-terminus
*Stab 25, Stab 26, and Stab 27 have three ribonucleotides at 5'-terminus
*Stab 29, Stab 30, and Stab 31, any purine at first three nucleotide positions from 5'-terminus are ribonucleotides
p = phosphorothioate linkage

Table V

A. 2.5 μmol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	6.5	163 μL	45 sec	2.5 min	7.5 min
S-Ethyl Tetrazole	23.8	238 μL	45 sec	2.5 min	7.5 min
Acetic Anhydride	100	233 μL	5 sec	5 sec	5 sec
N-Methyl Imidazole	186	233 μL	5 sec	5 sec	5 sec
TCA	176	2.3 mL	21 sec	21 sec	21 sec
Iodine	11.2	1.7 mL	45 sec	45 sec	45 sec
Beaucage	12.9	645 μL	100 sec	300 sec	300 sec
Acetonitrile	NA	6.67 mL	NA	NA	NA

B. 0.2 μmol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	15	31 μL	45 sec	233 sec	465 sec
S-Ethyl Tetrazole	38.7	31 μL	45 sec	233 min	465 sec
Acetic Anhydride	655	124 μL	5 sec	5 sec	5 sec
N-Methyl Imidazole	1245	124 μL	5 sec	5 sec	5 sec
TCA	700	732 μL	10 sec	10 sec	10 sec
Iodine	20.6	244 μL	15 sec	15 sec	15 sec
Beaucage	7.7	232 μL	100 sec	300 sec	300 sec
Acetonitrile	NA	2.64 mL	NA	NA	NA

C. 0.2 μmol Synthesis Cycle 96 well Instrument

Reagent	Equivalents:DNA/ 2'-O-methyl/Ribo	Amount: DNA/2'-O-methyl/Ribo	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time* Ribo
Phosphoramidites	22/33/66	40/60/120 μL	60 sec	180 sec	360sec
S-Ethyl Tetrazole	70/105/210	40/60/120 μL	60 sec	180 min	360 sec
Acetic Anhydride	265/265/265	50/50/50 μL	10 sec	10 sec	10 sec
N-Methyl Imidazole	502/502/502	50/50/50 μL	10 sec	10 sec	10 sec
TCA	238/475/475	250/500/500 μL	15 sec	15 sec	15 sec
Iodine	6.8/6.8/6.8	80/80/80 μL	30 sec	30 sec	30 sec
Beaucage	34/51/51	80/120/120	100 sec	200 sec	200 sec
Acetonitrile	NA	1150/1150/1150 μL	NA	NA	NA

- Wait time does not include contact time during delivery.
- Tandem synthesis utilizes double coupling of linker molecule

CLAIMS

What we claim is:

1. A multifunctional siNA molecule comprising a structure having Formula MF-III:

$$\begin{array}{cc} \mathbf{X} & \mathbf{X'} \\ \multicolumn{2}{c}{\mathbf{Y'\text{-}W\text{-}Y}} \end{array}$$

wherein

(a) each X, X', Y, and Y' is independently an oligonucleotide of length about 15 nucleotides to about 50 nucleotides;

(b) X comprises nucleotide sequence that is complementary to nucleotide sequence present in region Y';

(c) X' comprises nucleotide sequence that is complementary to nucleotide sequence present in region Y;

(d) each X and X' is independently of length sufficient to stably interact with a first and a second HCV target nucleic acid sequence, respectively, or a portion thereof;

(e) W represents a nucleotide or non-nucleotide linker that connects sequences Y' and Y; and

(f) said multifunctional siNA directs cleavage of the first and second HCV target sequence via RNA interference.

2. The multifunctional siNA molecule of claim 1, wherein W connects the 3'-end of sequence Y' with the 3'-end of sequence Y.

3. The multifunctional siNA molecule of claim 1, wherein W connects the 3'-end of sequence Y' with the 5'-end of sequence Y.

4. The multifunctional siNA molecule of claim 1, wherein W connects the 5'-end of sequence Y' with the 5'-end of sequence Y.

5. The multifunctional siNA molecule of claim 1, wherein W connects the 5'-end of sequence Y' with the 3'-end of sequence Y.

6. The multifunctional siNA molecule of claim 1, wherein a terminal phosphate group is present at the 5'-end of any of sequence X, X', Y, or Y'.

7. The multifunctional siNA molecule of claim 1, wherein W connects sequences Y and Y' via a biodegradable linker.

8. The multifunctional siNA molecule of claim 1, wherein W further comprises a conjugate, label, aptamer, ligand, lipid, or polymer.

9. The multifunctional siNA molecule of claim 1, wherein any of sequence X, X', Y, or Y' comprises a 3'-terminal cap moiety.

10. The multifunctional siNA molecule of claim 9, wherein said terminal cap moiety is an inverted deoxyabasic moiety.

11. The multifunctional siNA molecule of claim 10, wherein said terminal cap moiety is an inverted deoxynucleotide moiety.

12. The multifunctional siNA molecule of claim 10, wherein said terminal cap moiety is a dinucleotide moiety.

13. The multifunctional siNA molecule of claim 12, wherein said dinucleotide is dithymidine (TT).

14. The multifunctional siNA molecule of claim 1, wherein said siNA molecule comprises no ribonucleotides.

15. The multifunctional siNA molecule of claim 1, wherein said siNA molecule comprises one or more ribonucleotides.

16. The multifunctional siNA molecule of claim 1, wherein any purine nucleotide in said siNA is a 2'-O-methyl purine nucleotide.

17. The multifunctional siNA molecule of claim 1, wherein any purine nucleotide in said siNA is a 2'-deoxy purine nucleotide.

18. The multifunctional siNA molecule of claim 1, wherein any pyrimidine nucleotide in said siNA is a 2'-deoxy-2'-fluoro pyrimidine nucleotide.

19. The multifunctional siNA molecule of claim 1, wherein each X, X', Y, and Y' independently comprises about 19 to about 23 nucleotides.

20. The multifunctional siNA molecule of claim 1, wherein said first and second HCV target sequence each is a HCV RNA sequence.

21. The multifunctional siNA molecule of claim 1, wherein said first HCV target sequence is a HCV RNA sequence, and said second HCV target sequence is a cellular target RNA sequence that is required for HCV infection or replication.

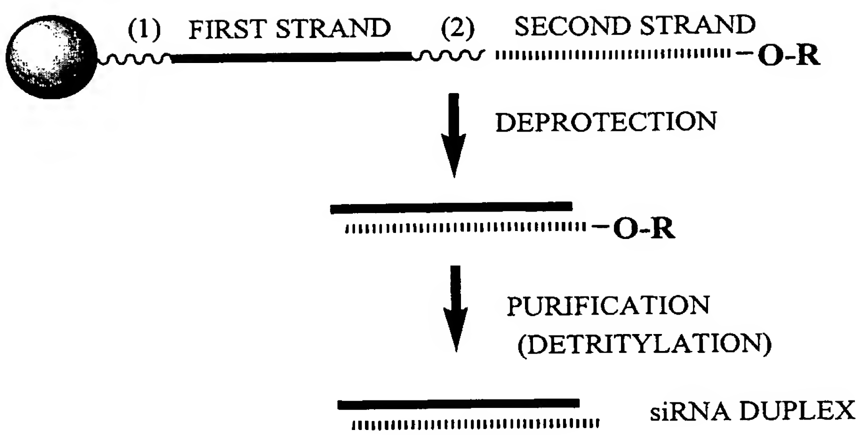
22. The multifunctional siNA molecule of claim 1, wherein said first HCV target sequence is a cellular target RNA sequence that is required for HCV infection or replication, and said second HCV target sequence is a HCV RNA sequence.

23. The multifunctional siNA molecule of claim 1, wherein said first and second HCV target sequences are each a cellular target RNA sequence that is required for HCV infection or replication.

24. The multifunctional siNA molecule of claim 21 or claim 22, wherein said cellular target RNA sequence is selected from a La antigen, FAS, FAS ligand, interferon regulatory factor, cellular PKR protein, elF2Bgamma, elF2gamma, human DEAD Box protein (DDX3), and polypyrimidine tract-binding protein.

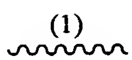
25. A pharmaceutical composition comprising the multifunctional siNA molecule of claim 1 and an acceptable carrier or diluent.

Figure 1

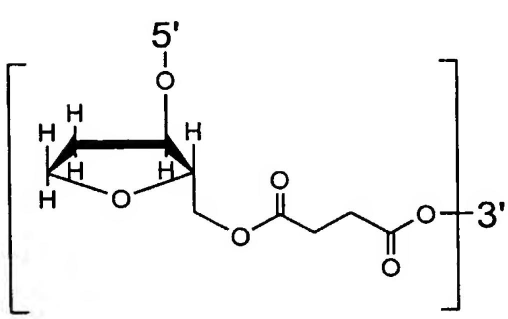


 = SOLID SUPPORT

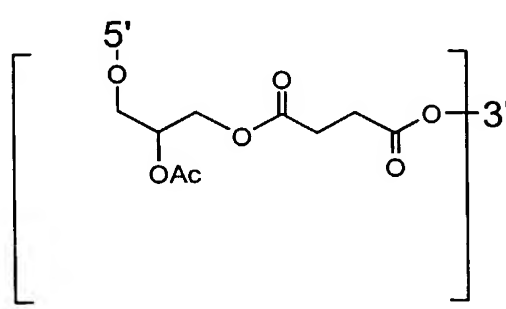
R = TERMINAL PROTECTING GROUP
FOR EXAMPLE:
DIMETHOXYTRITYL (DMT)

 = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)

 = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)



INVERTED DEOXYABASIC SUCCINATE LINKAGE



GLYCERYL SUCCINATE LINKAGE

Figure 2

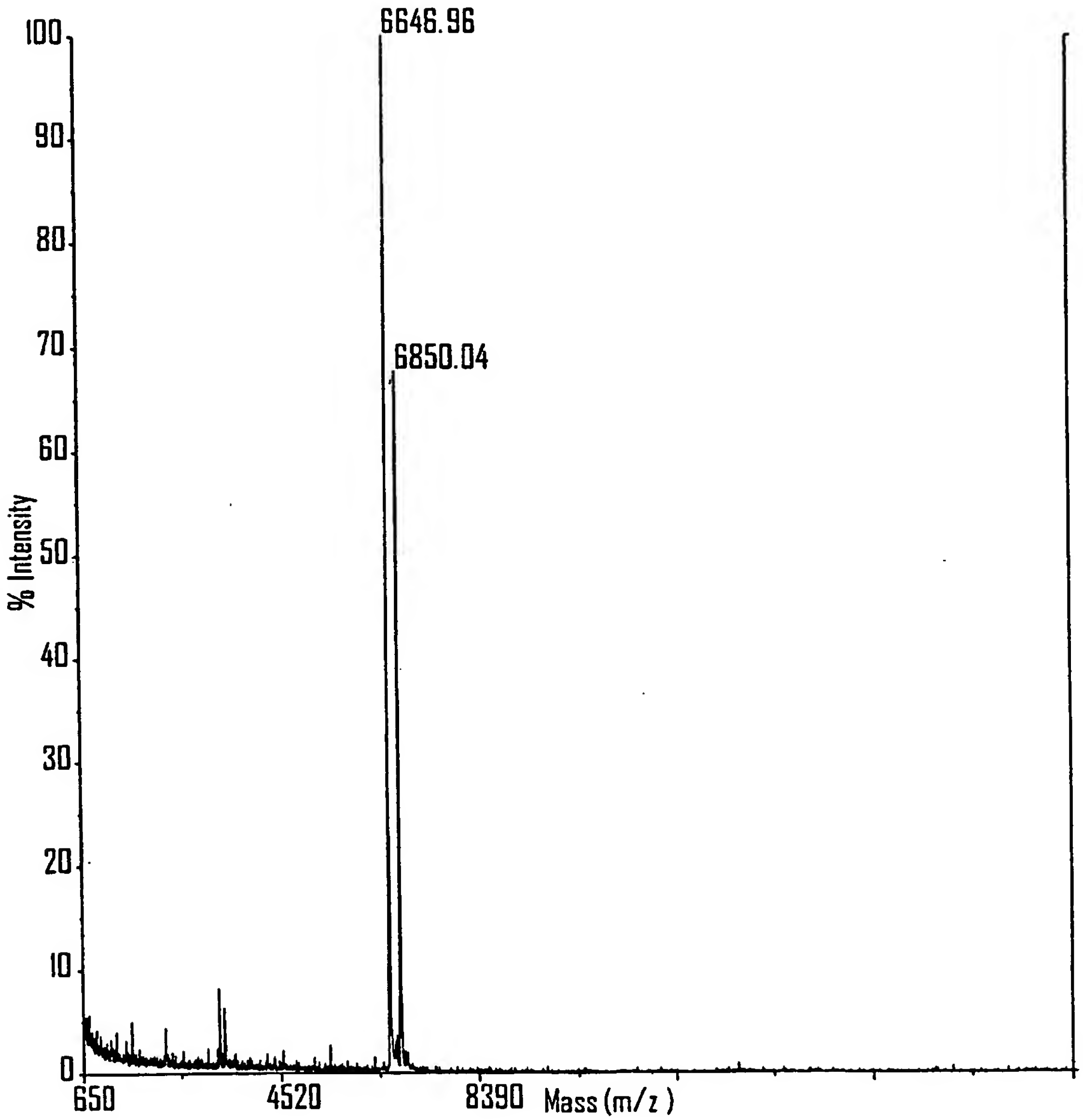

6646.96
6850.04
100
90
80
70
60
50
40
30
20
10
0
% Intensity
650
4520
8390
Mass (m/z)

Figure 3

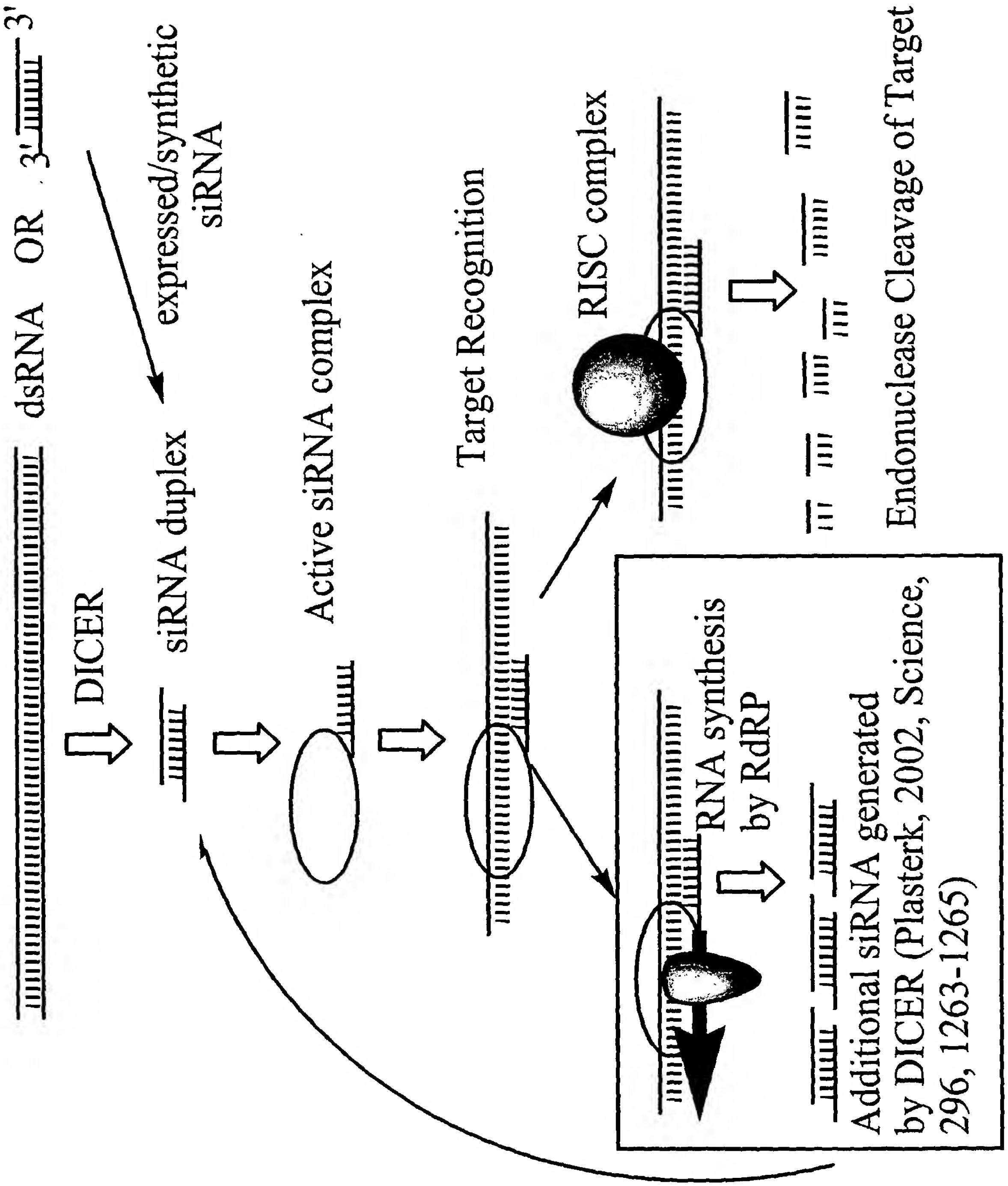

dsRNA OR 3' 3'
DICER
siRNA duplex
expressed/synthetic siRNA
Active siRNA complex
Target Recognition
RISC complex
Endonuclease Cleavage of Target
RNA synthesis by RdRP
Additional siRNA generated by DICER (Plasterk, 2002, Science, 296, 1263-1265)

Figure 4

A

SENSE STRAND (SEQ ID NO 2010)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 2011)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

B

SENSE STRAND (SEQ ID NO 2012)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-OM EXCEPT POSITIONS (N N)

5'- N N N N N N N N N N N N N N N N N N N (N_sN) -3'
3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 2013)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

C

SENSE STRAND (SEQ ID NO 2014)
ALL PYRIMIDINES = 2'-O-ME OR 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 2015)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

D

SENSE STRAND (SEQ ID NO 2016)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 2013)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

E

SENSE STRAND (SEQ ID NO 2017)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 2013)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

F

SENSE STRAND (SEQ ID NO 2016)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 2018)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTIDES (eg. THYMIDINE) OR UNIVERSAL BASES

B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT

L = GLYCERYL or B THAT IS OPTIONALLY PRESENT

S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE that is optionally absent

Figure 5

A

SENSE STRAND (SEQ ID NO 2019)

5'- B- G C G G G C C U U G C C U A C U A U U *T T*-B -3'
3'- L-*T*$_S$*T* C G C C C G G A A C G G A U G A U A A -5'

ANTISENSE STRAND (SEQ ID NO 2020)

B

SENSE STRAND (SEQ ID NO 2021)

5'- g c g g g c c u u g c c u a c u a u u *T*$_S$*T* -3'
3'- L-*T*$_S$*T* c g c c c g g a a c g g a u g a u a a -5'

ANTISENSE STRAND (SEQ ID NO 2022)

C

SENSE STRAND (SEQ ID NO 2023)

5'- B-G c G G G c c u u G c c u A c u A u u *T T*-B -3'
3'- L-*T*$_S$*T* *c* G *c c c* G G A A *c* G G A *u* G A *u* A A -5'

ANTISENSE STRAND (SEQ ID NO 2024)

D

SENSE STRAND (SEQ ID NO 2025)

5'- B-*G* c *G G G* c c u u *G* c c u *A* c u *A* u u *T T*-B -3'
3'- L-*T*$_S$*T* c g c c c g g a a c g g a *u* g a *u* a a -5'

ANTISENSE STRAND (SEQ ID NO 2022)

E

SENSE STRAND (SEQ ID NO 2026)

5'- B-G *c* G G G *c c u u* G *c c u* A *c u* A *u u* *T T*-B -3'
3'- L-*T*$_S$*T* c g c c c g g a a c g g a *u* g a *u* a a -5'

ANTISENSE STRAND (SEQ ID NO 2022)

F

SENSE STRAND (SEQ ID NO 2025)

5'- B-*G* c *G G G* c c u u *G* c c u *A* c u *A* u u *T T*-B -3'
3'- L-*T*$_S$*T* c *G* c c c *G G A A* c *G G A* u *G A* u *A A* -5'

ANTISENSE STRAND (SEQ ID NO 2027)

lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro
italic lower case = 2'-deoxy-2'-fluoro
underline = 2'-O-methyl

ITALIC UPPER CASE = DEOXY
B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT
L = GLYCERYL MOIETY or B OPTIONALLY PRESENT
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE OPTIONALLY PRESENT

Figure 6

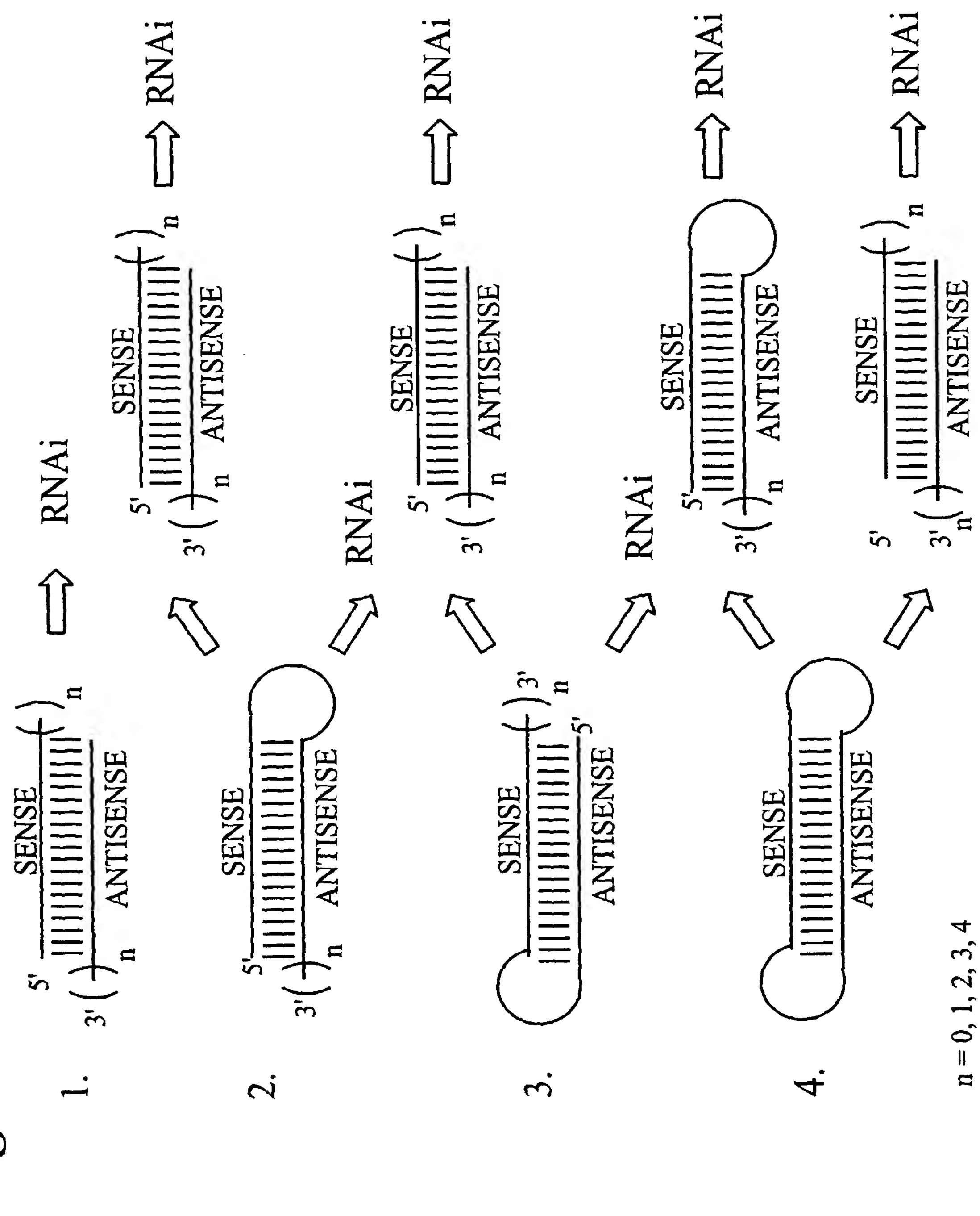


Figure 7

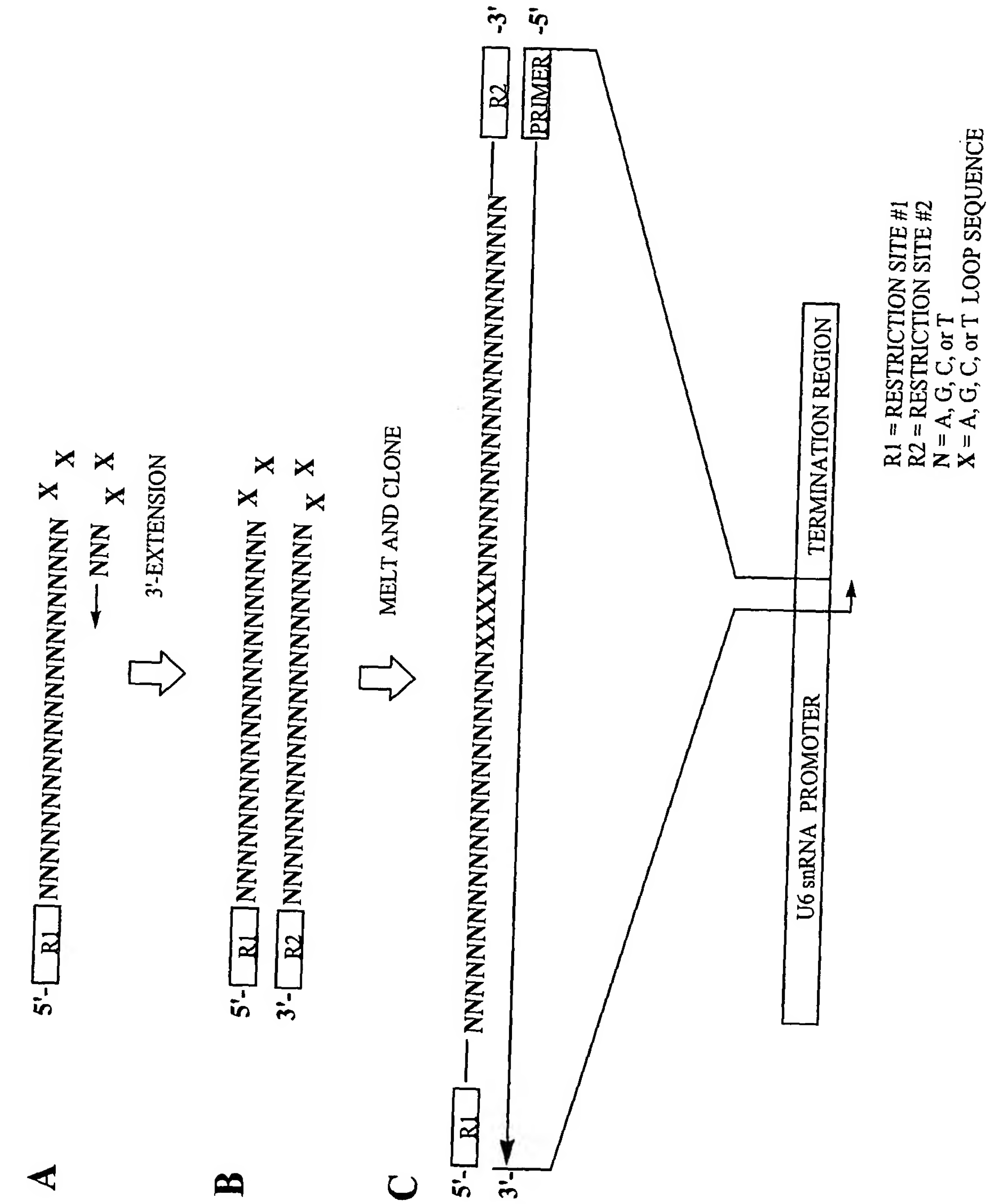

A
5'- R1 NNNNNNNNNNNNNNNNNN X X X X
NNN
3'-EXTENSION
B
5'- R1 NNNNNNNNNNNNNNNNNN X X
3'- R2 NNNNNNNNNNNNNNNNNN X X
MELT AND CLONE
C
5'- R1 NNNNNNNNNNNNNNNNNNNXXXXNNNNNNNNNNNNNNNNNN R2 -3'
3'- PRIMER -5'
U6 snRNA PROMOTER
TERMINATION REGION
R1 = RESTRICTION SITE #1
R2 = RESTRICTION SITE #2
N = A, G, C, or T
X = A, G, C, or T LOOP SEQUENCE

Figure 8

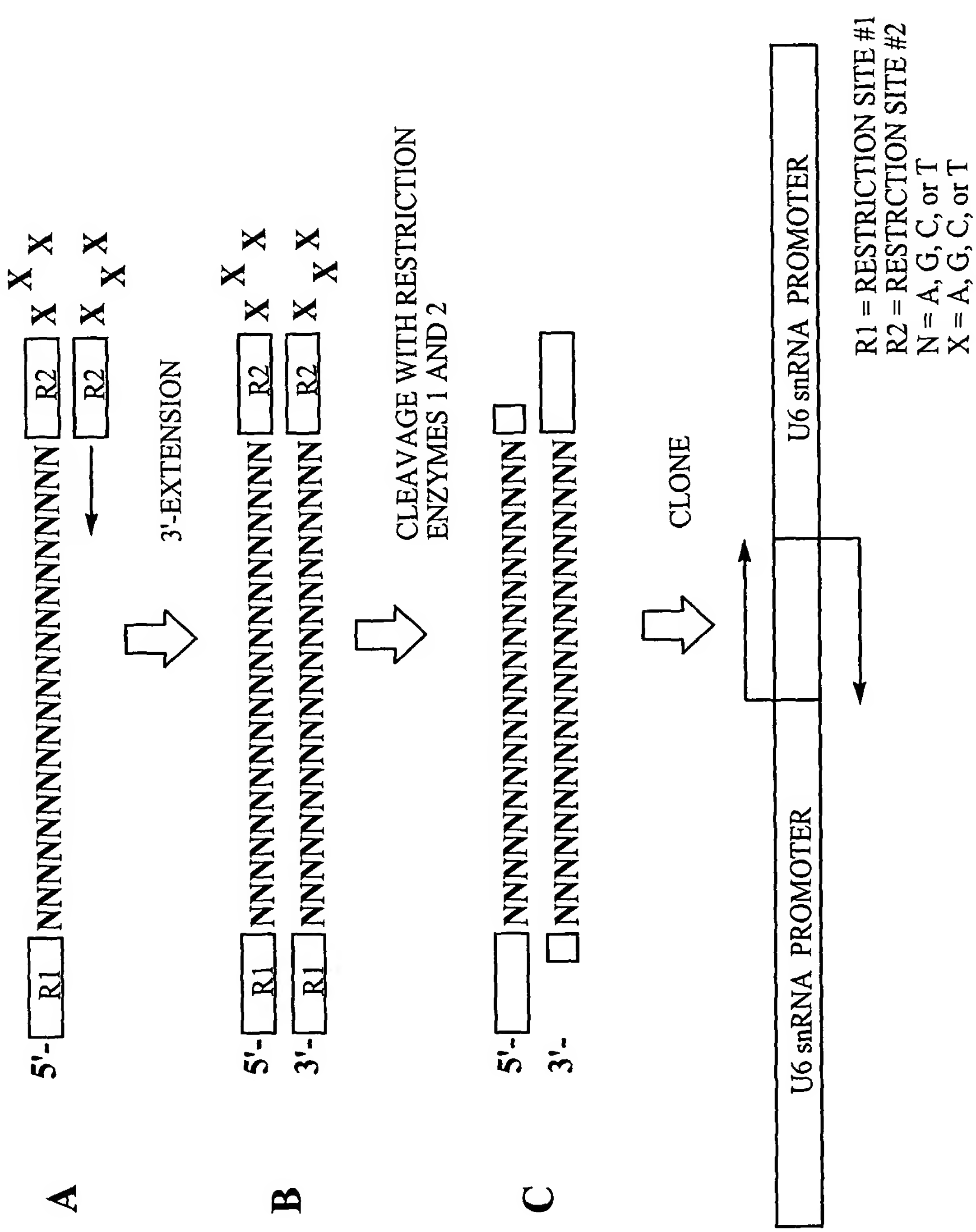

A
5'- R1 NNNNNNNNNNNNNNNNNNN R2 X X X X
R2 X X X X
3'-EXTENSION
B
5'- R1 NNNNNNNNNNNNNNNNNNN R2 X X X X
3'- R1 NNNNNNNNNNNNNNNNNNN R2 X X X X
CLEAVAGE WITH RESTRICTION ENZYMES 1 AND 2
C
5'- NNNNNNNNNNNNNNNNNNN
3'- NNNNNNNNNNNNNNNNNNN
CLONE
U6 snRNA PROMOTER
U6 snRNA PROMOTER
R1 = RESTRICTION SITE #1
R2 = RESTRCTION SITE #2
N = A, G, C, or T
X = A, G, C, or T

Figure 9: Target site Selection using siRNA

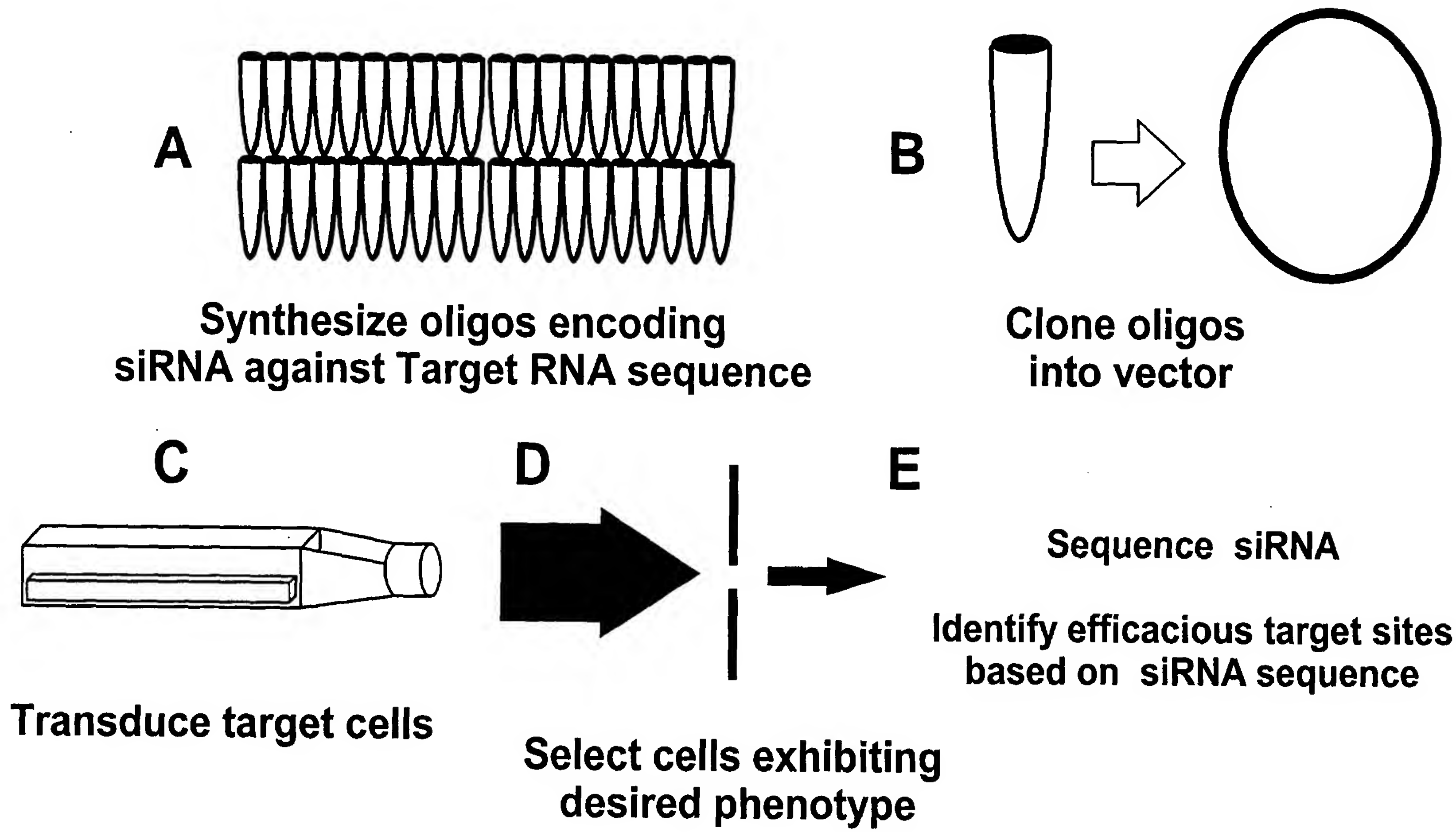
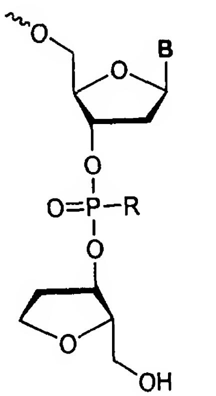
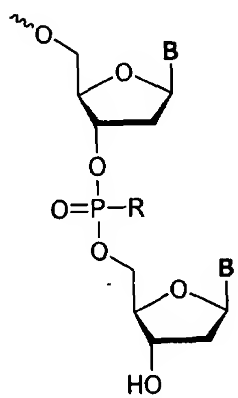


Figure 10



1



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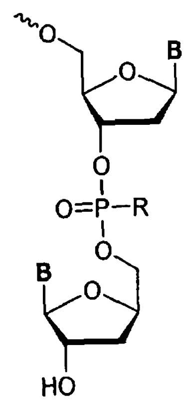

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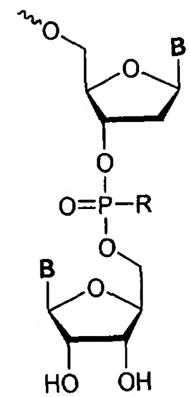

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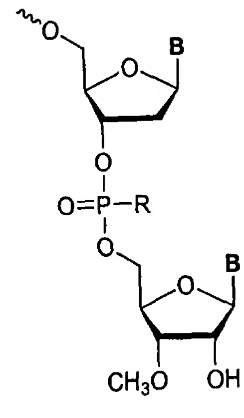
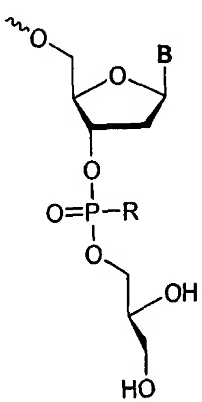


5



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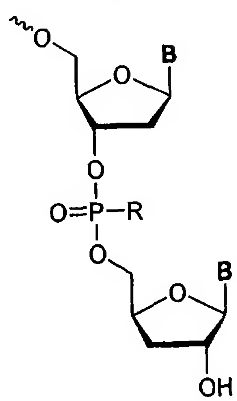

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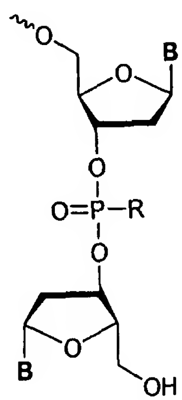

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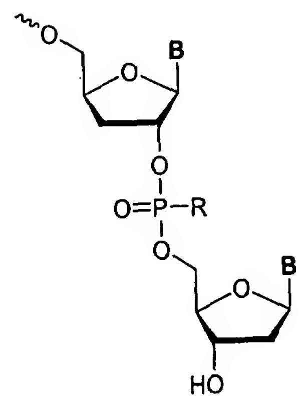
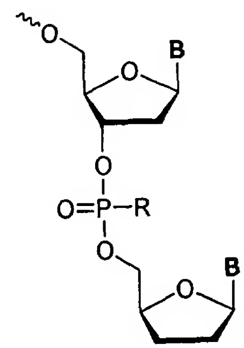


9



10

R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl
B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

Figure 11: Modification Strategy

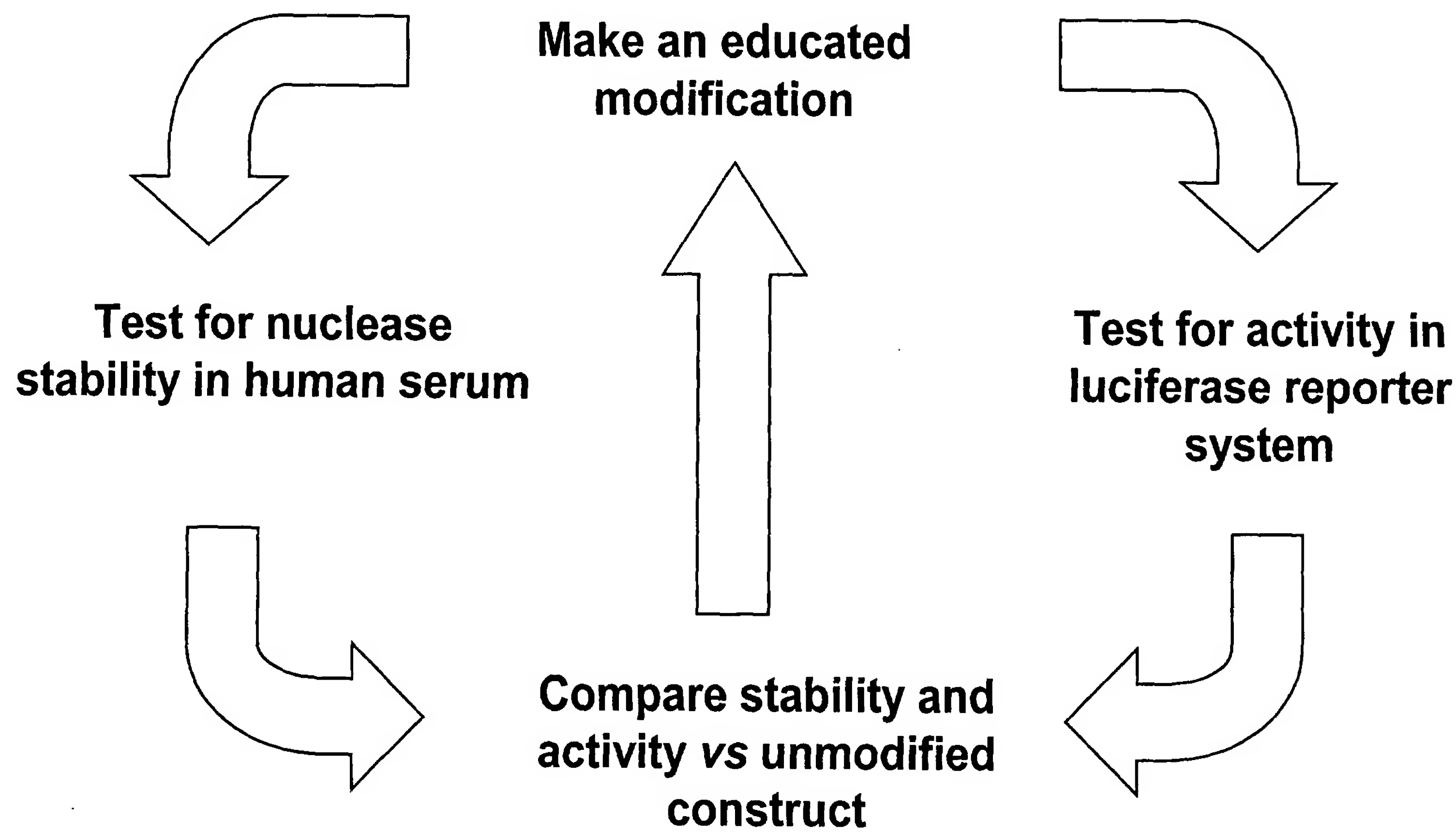


Figure 12: Phosphorylated siNA constructs

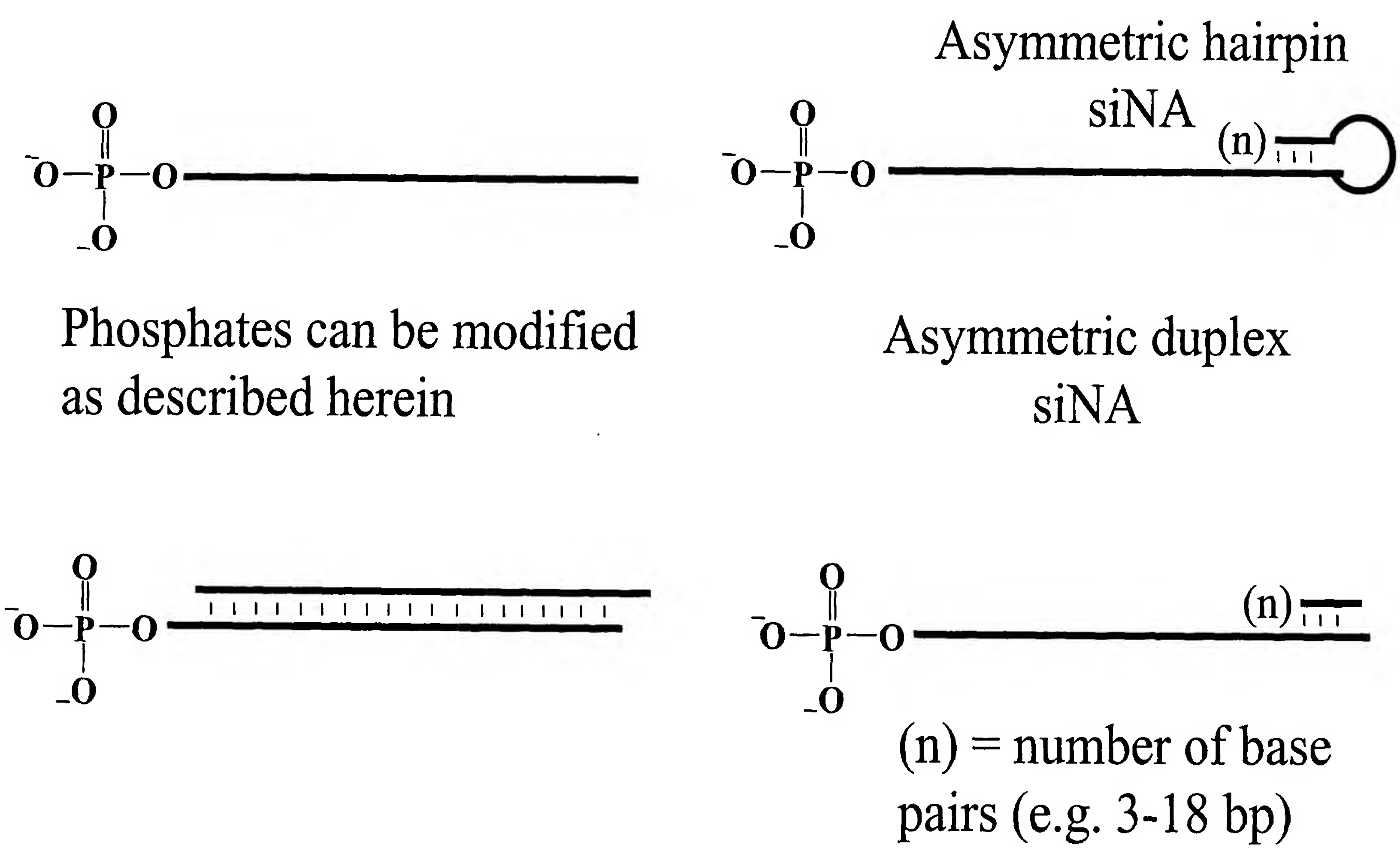


Figure 13: 5'-phosphate modifications

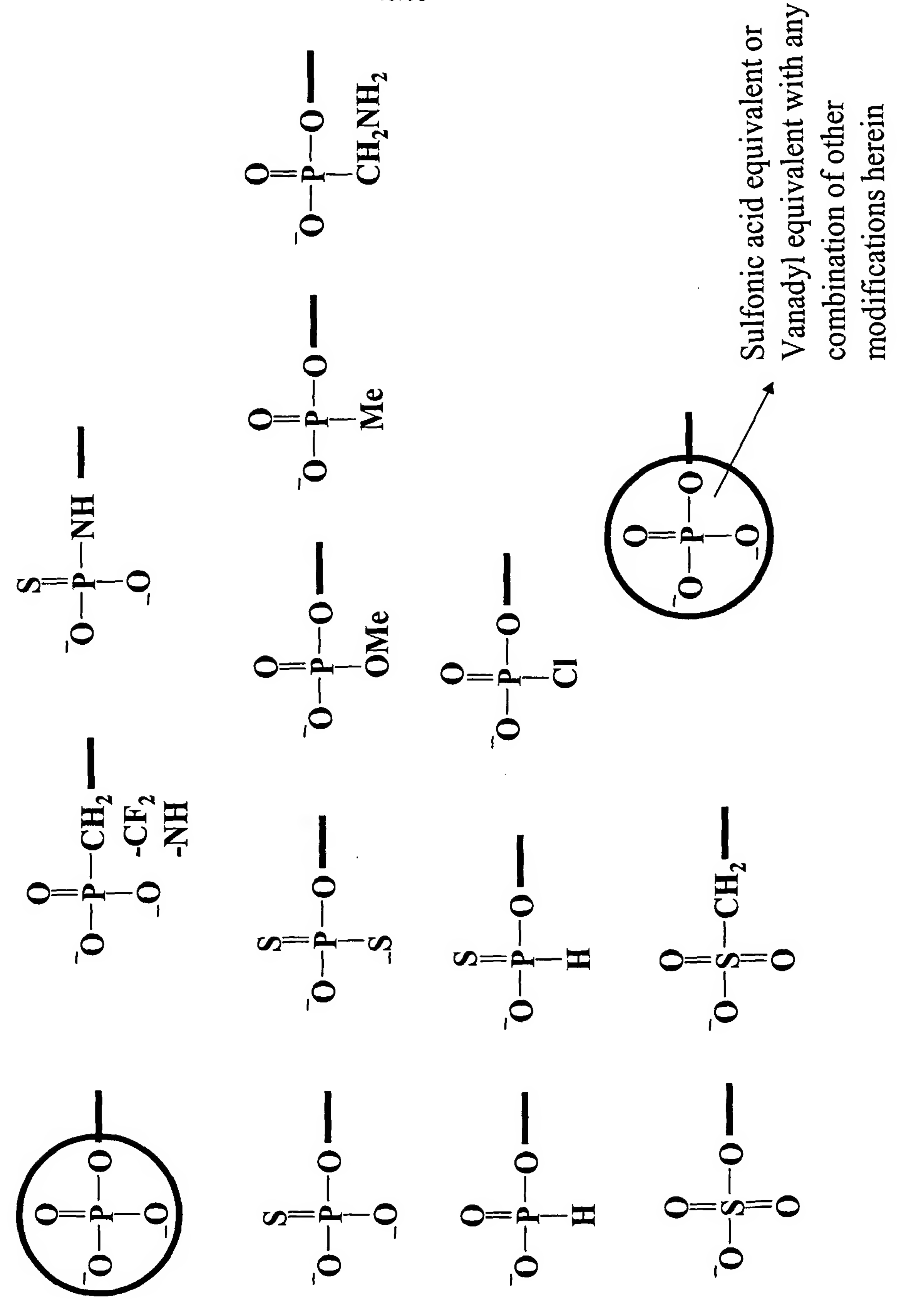
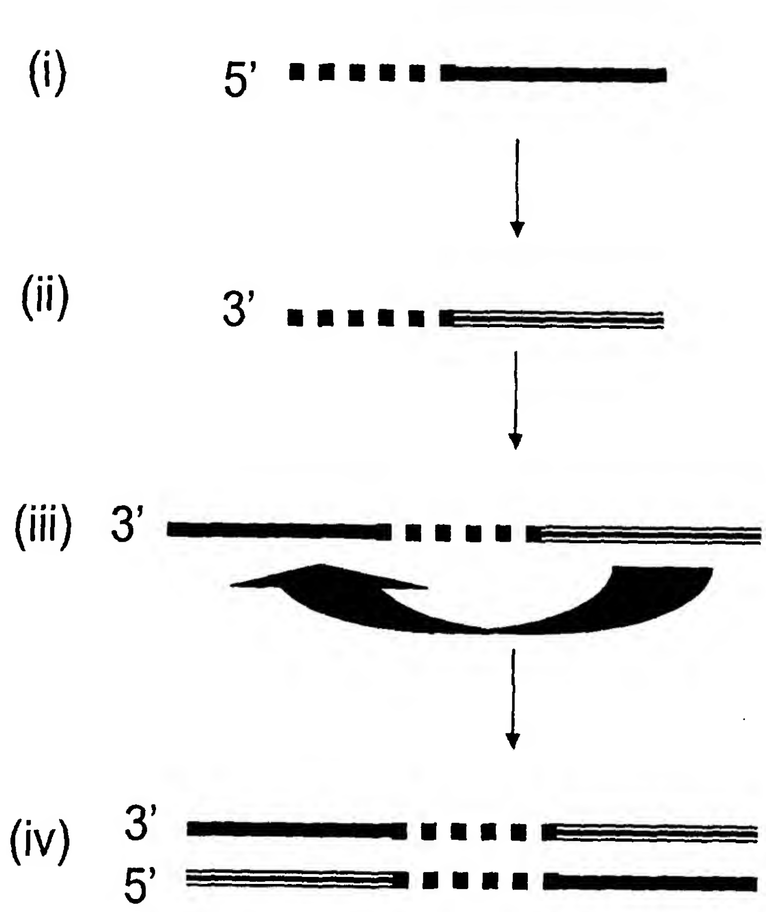


Figure 14A: Duplex forming oligonucleotide constructs that utilize Palindrome or repeat sequences



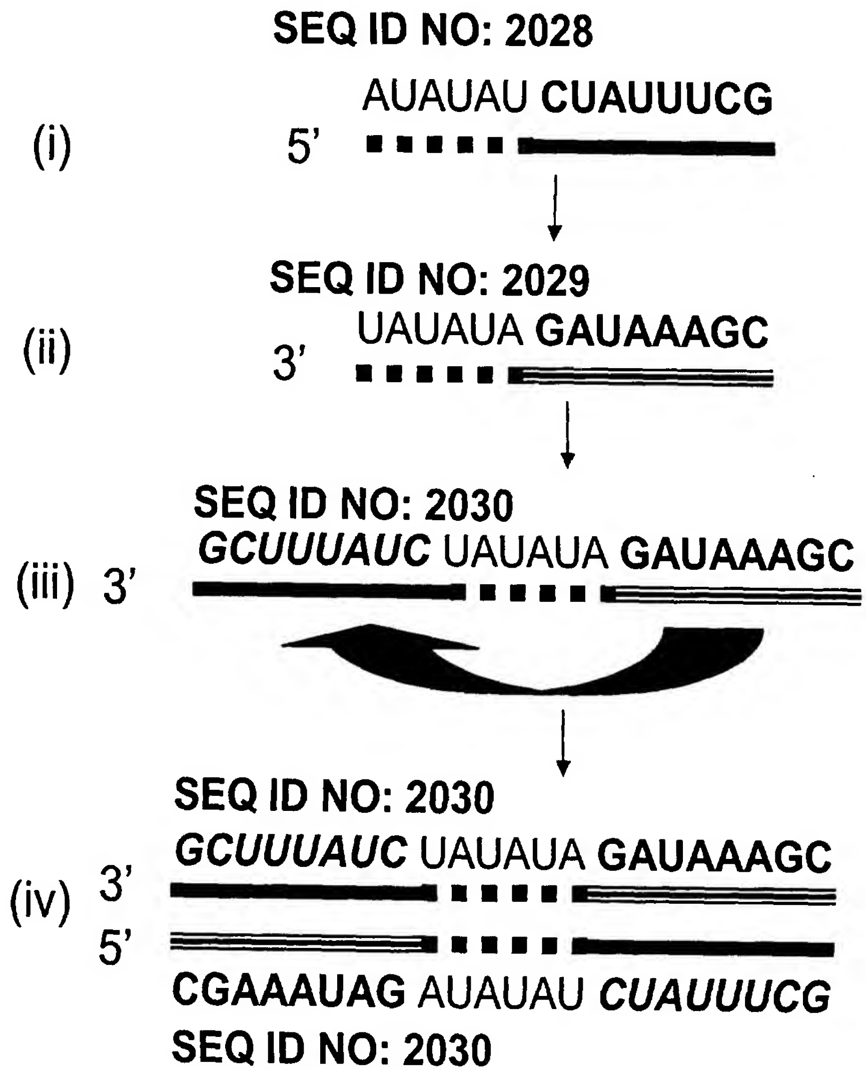
(i) Identify Target Nucleic Acid sequence (e.g., 14 to 24 nucleotides in length) containing palindrome/repeat sequence at 5'-end (dashed portion)

(ii) Design Complementary Sequence to the Target Nucleic Acid sequence of (i) above

(iii) Append inverse sequence of the Non-palindromic Complementary Sequence of (ii) to 3'-end of complementary sequence

(iv) Self assembly of self complementary strands to form duplex construct

Figure 14B: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence



(i) Identify Target Nucleic Acid sequence (e.g., 14 to 24 nucleotides in length) containing palindrome/repeat sequence at 5'-end (dashed portion)

(ii) Design Complementary Sequence to the Target Nucleic Acid sequence of (i) above

(iii) Append inverse sequence of the Non-palindromic Complementary Sequence of (ii) to 3'-end of complementary sequence

(iv) Self assembly of self complementary strands to form duplex construct (blunt ends)

Figure 14C: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly

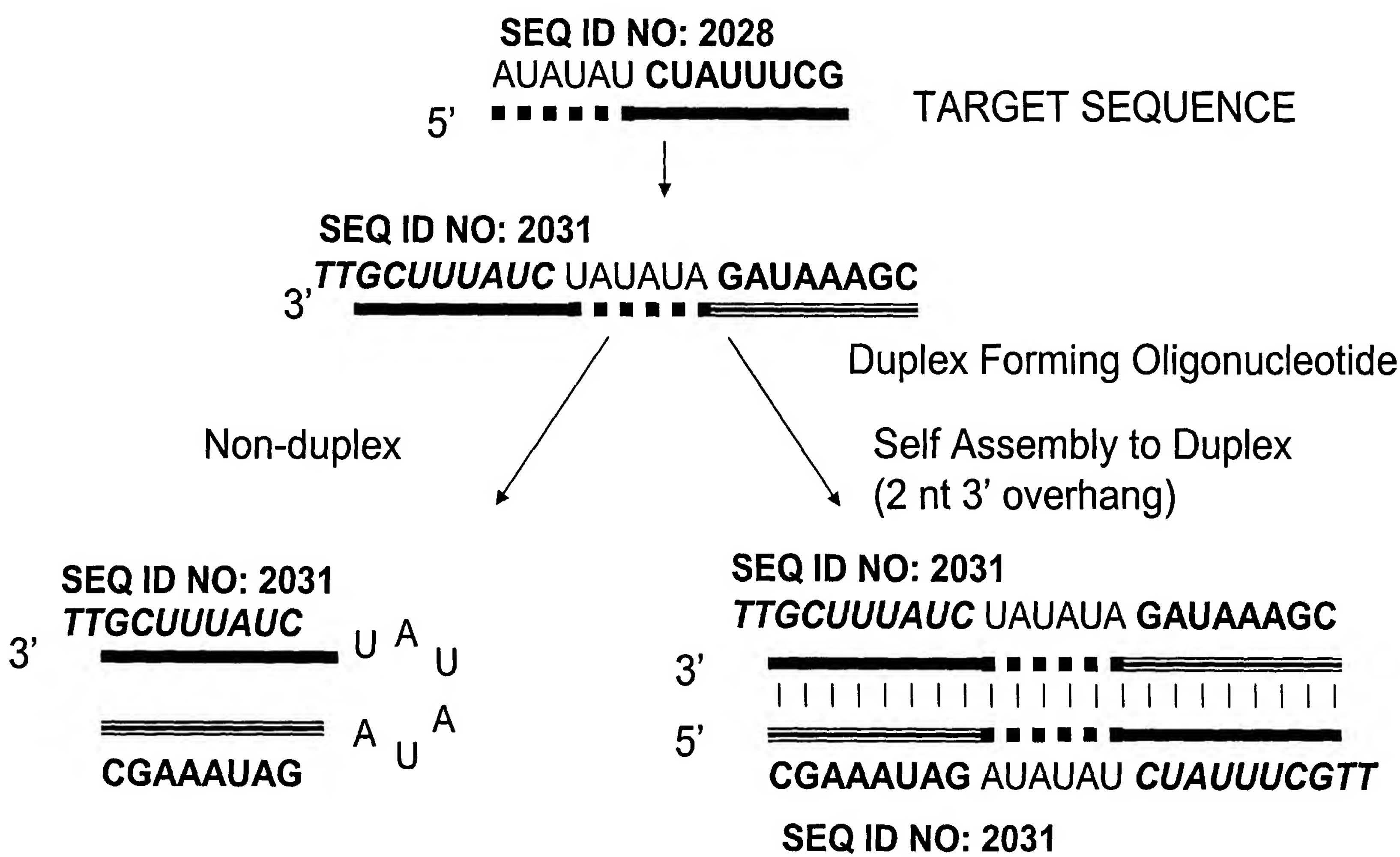


***Figure 14D:* Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly and inhibition of Target Sequence Expression**

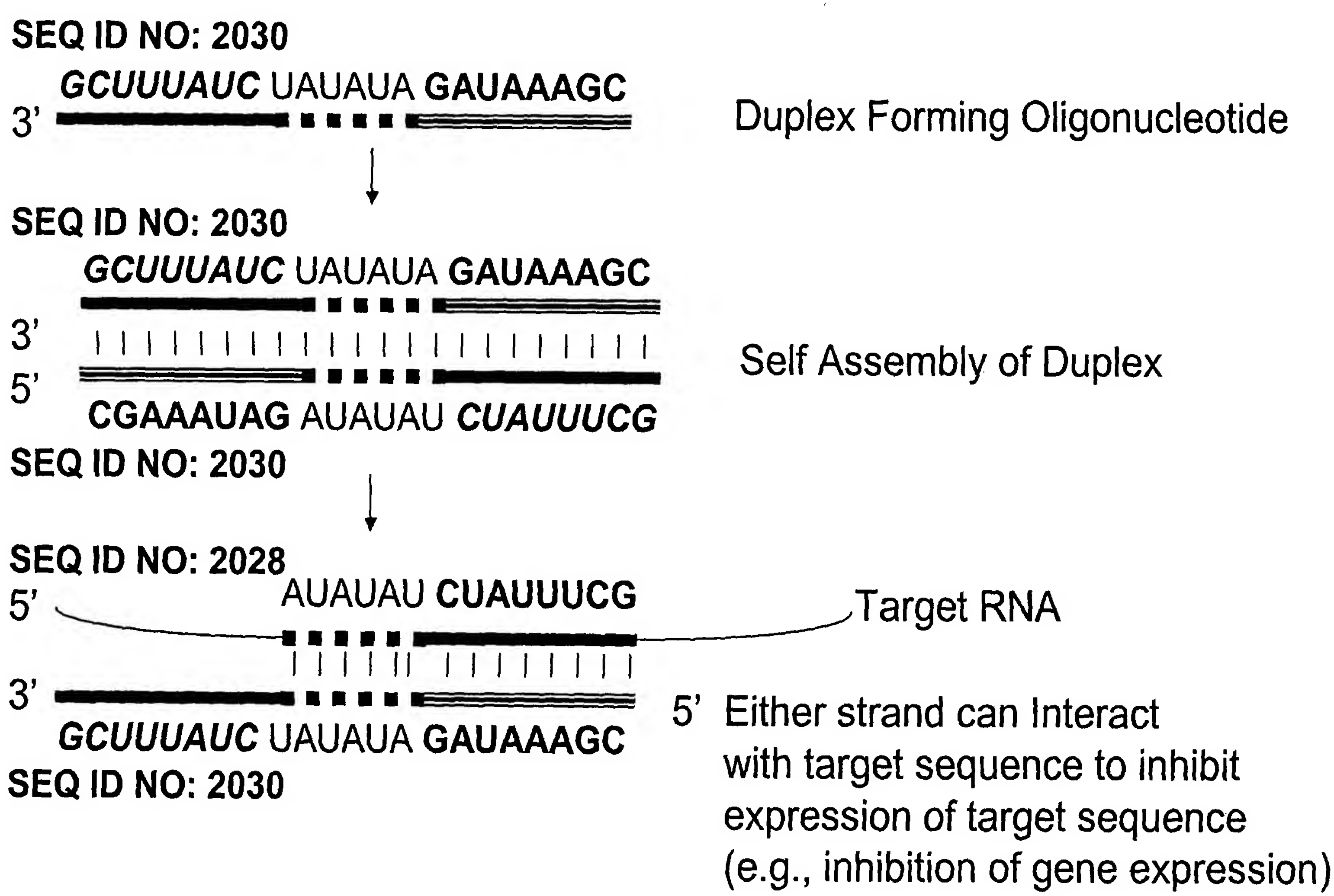
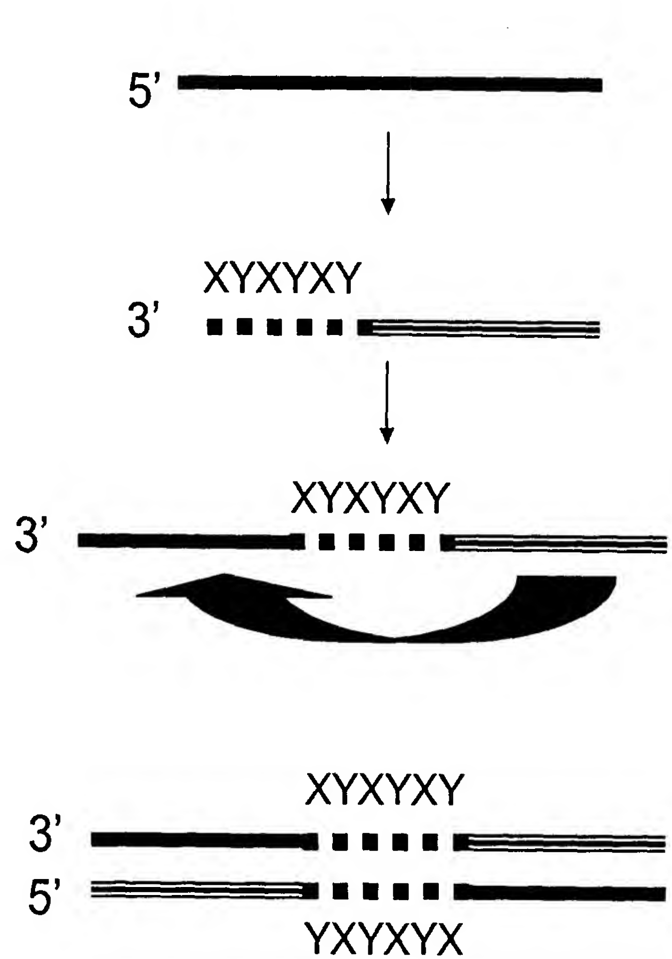


Figure 15: Duplex forming oligonucleotide constructs that utilize artificial palindrome or repeat sequences



Identify Target Nucleic Acid sequence (e.g., 14 to 24 nucleotides in length)

Design Complementary Sequence and utilize modified nucleotides (shown as X, Y) that interact with a portion of the target sequence and result in the formation of a palindrome/repeat sequence (e.g., 2 to 12 nucleotides) at 3'-end (dashed portion)

Append inverse sequence of Complementary region to 3'-end of palindrome/repeat sequence

Hybridize self complementary strands to form duplex siNA construct

Figure 16: Examples of double stranded multifunctional siNA constructs with distinct complementary regions

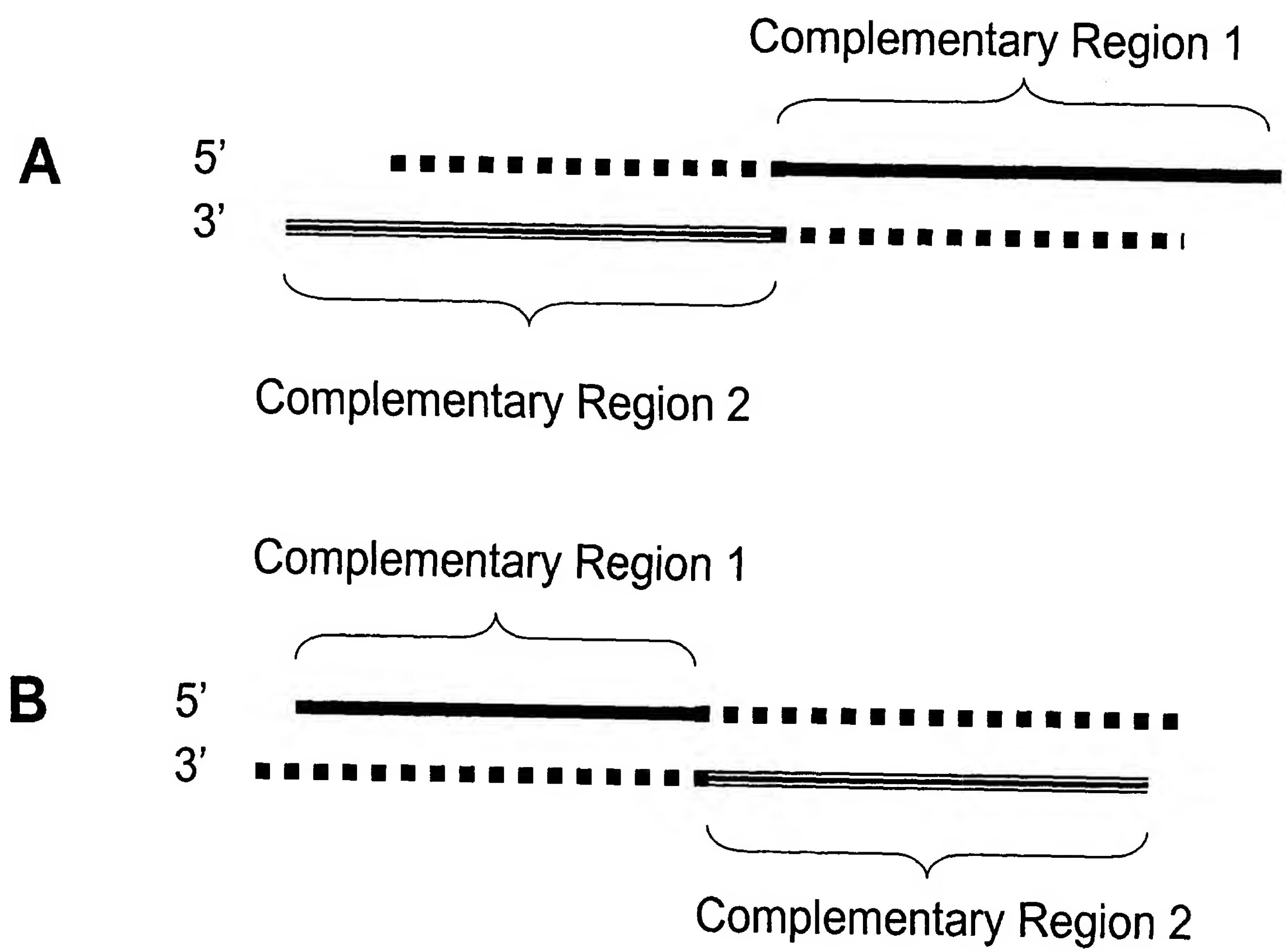


Figure 17: Examples of hairpin multifunctional siNA constructs with distinct complementary regions

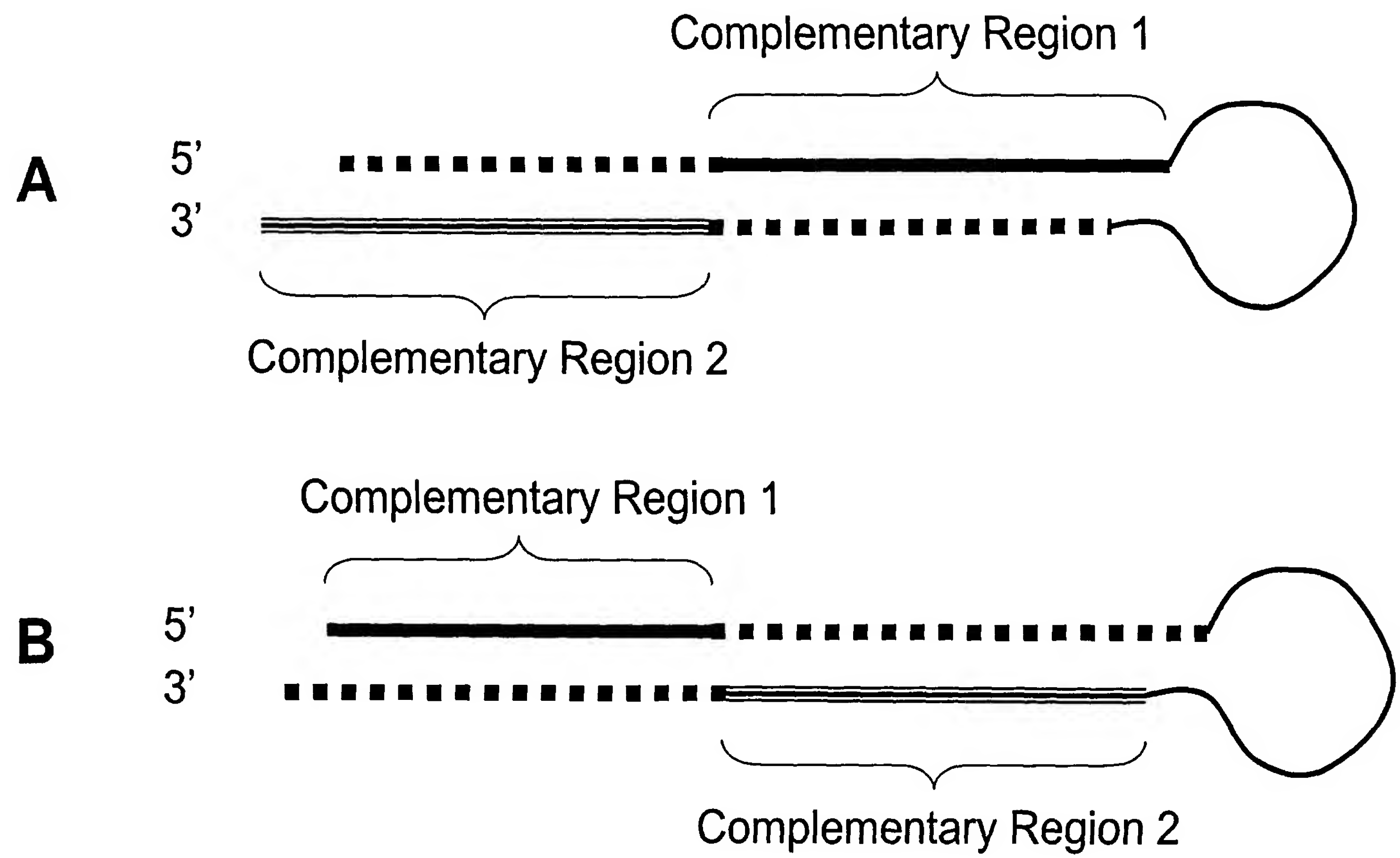


Figure 18: Examples of double stranded multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region

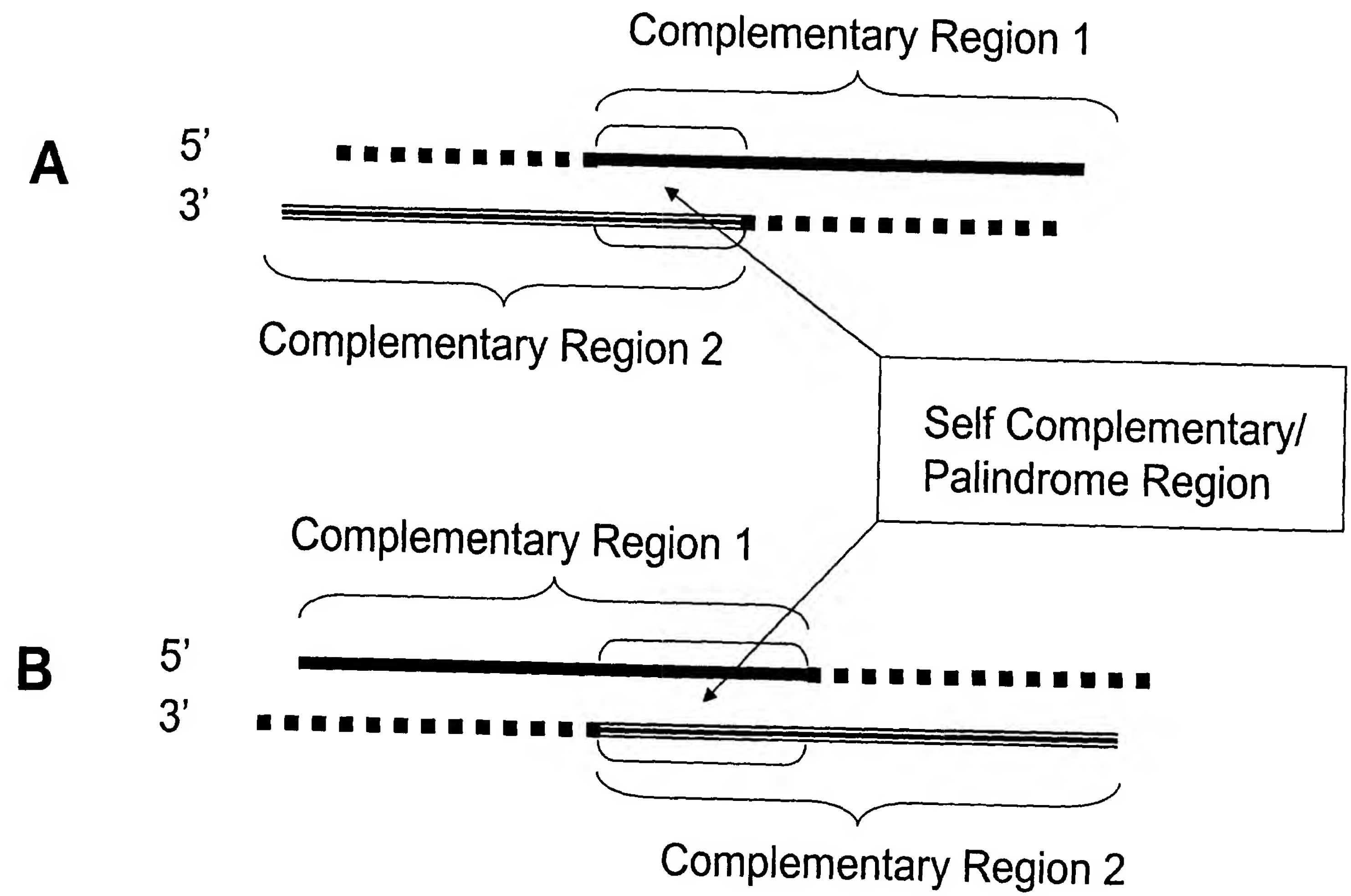


Figure 19: Examples of hairpin multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region

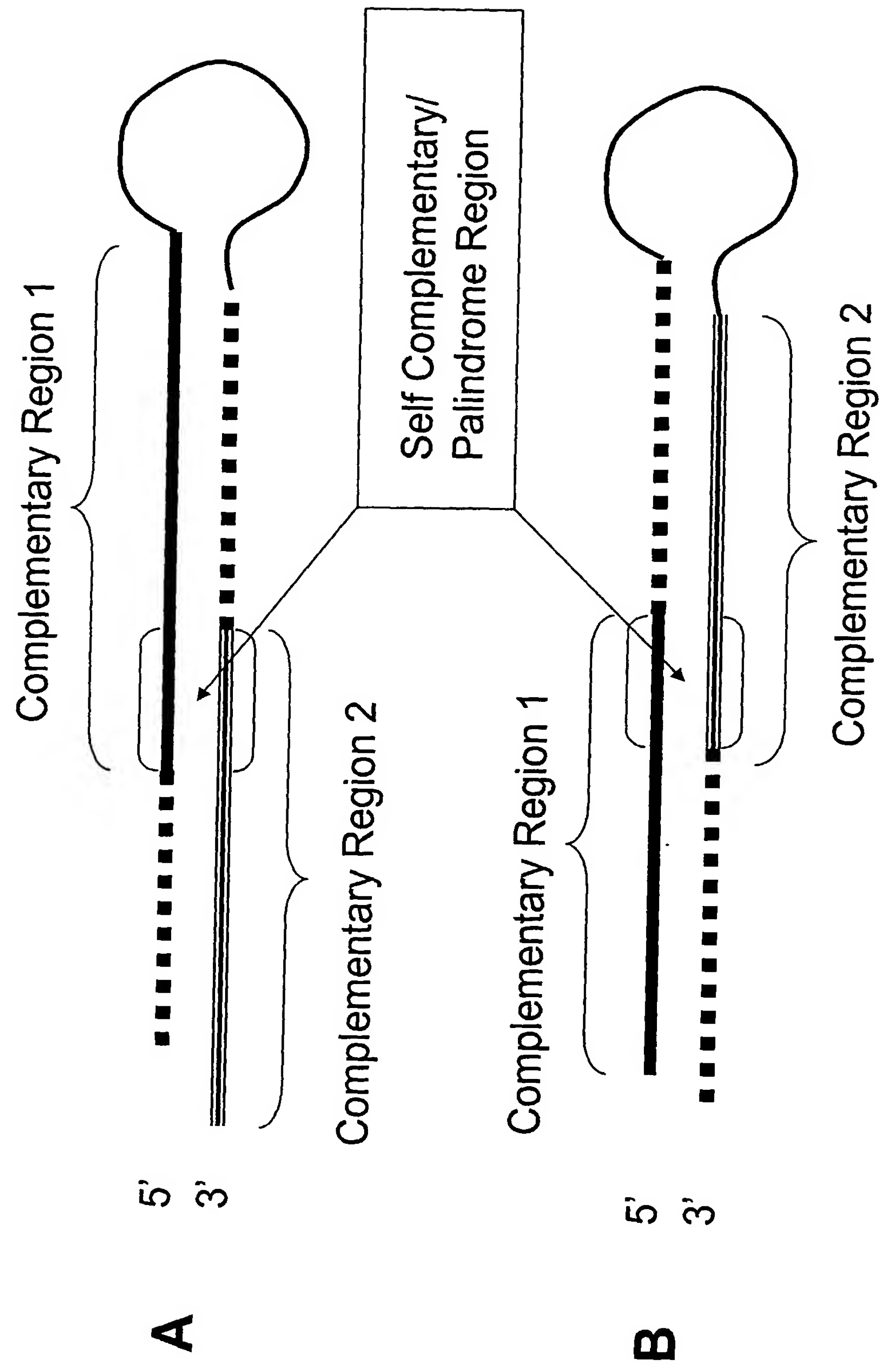


Figure 20: Example of multifunctional siNA targeting two Separate Target nucleic acid sequences

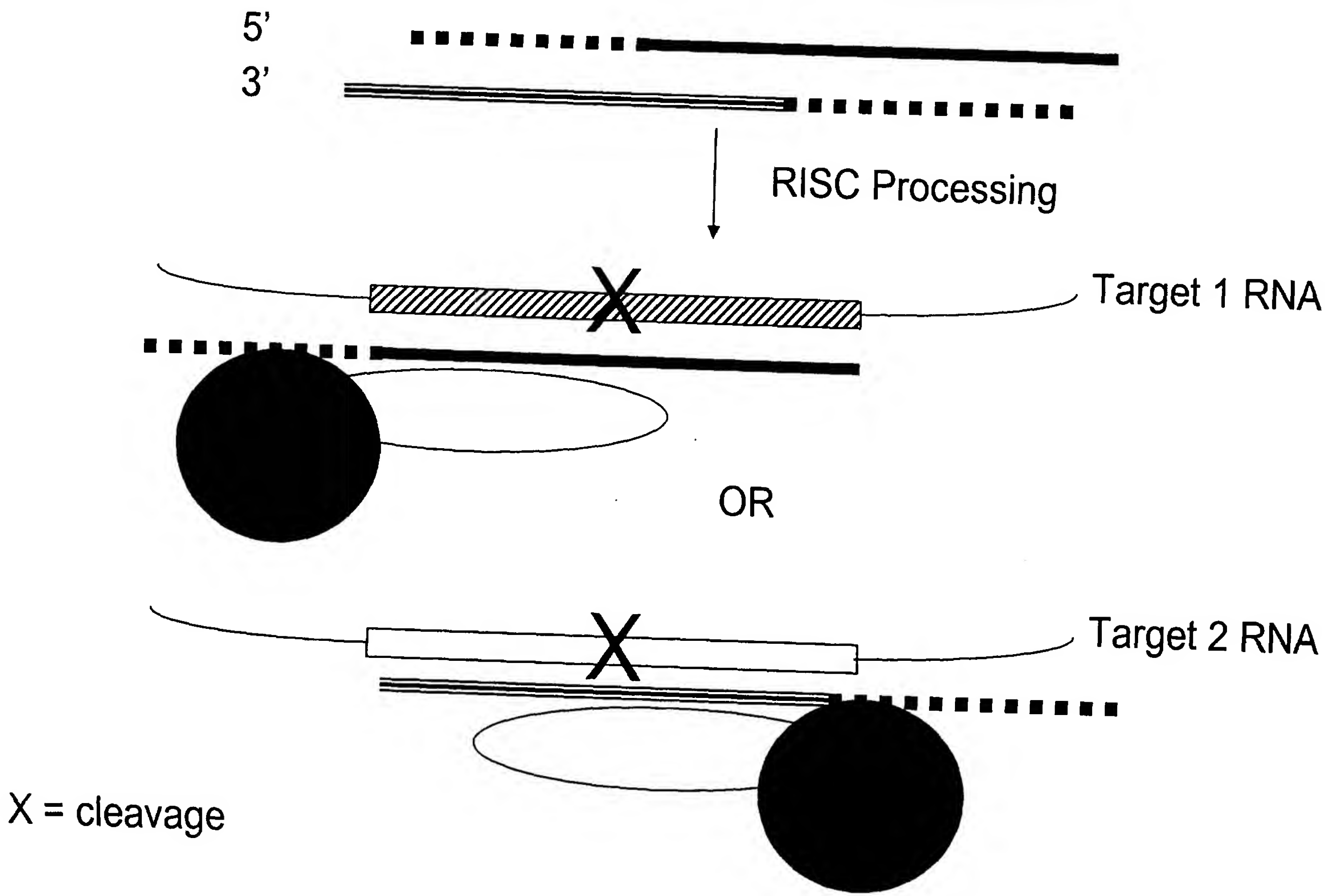


Figure 21: Example of multifunctional siNA targeting two regions within the same target nucleic acid sequence

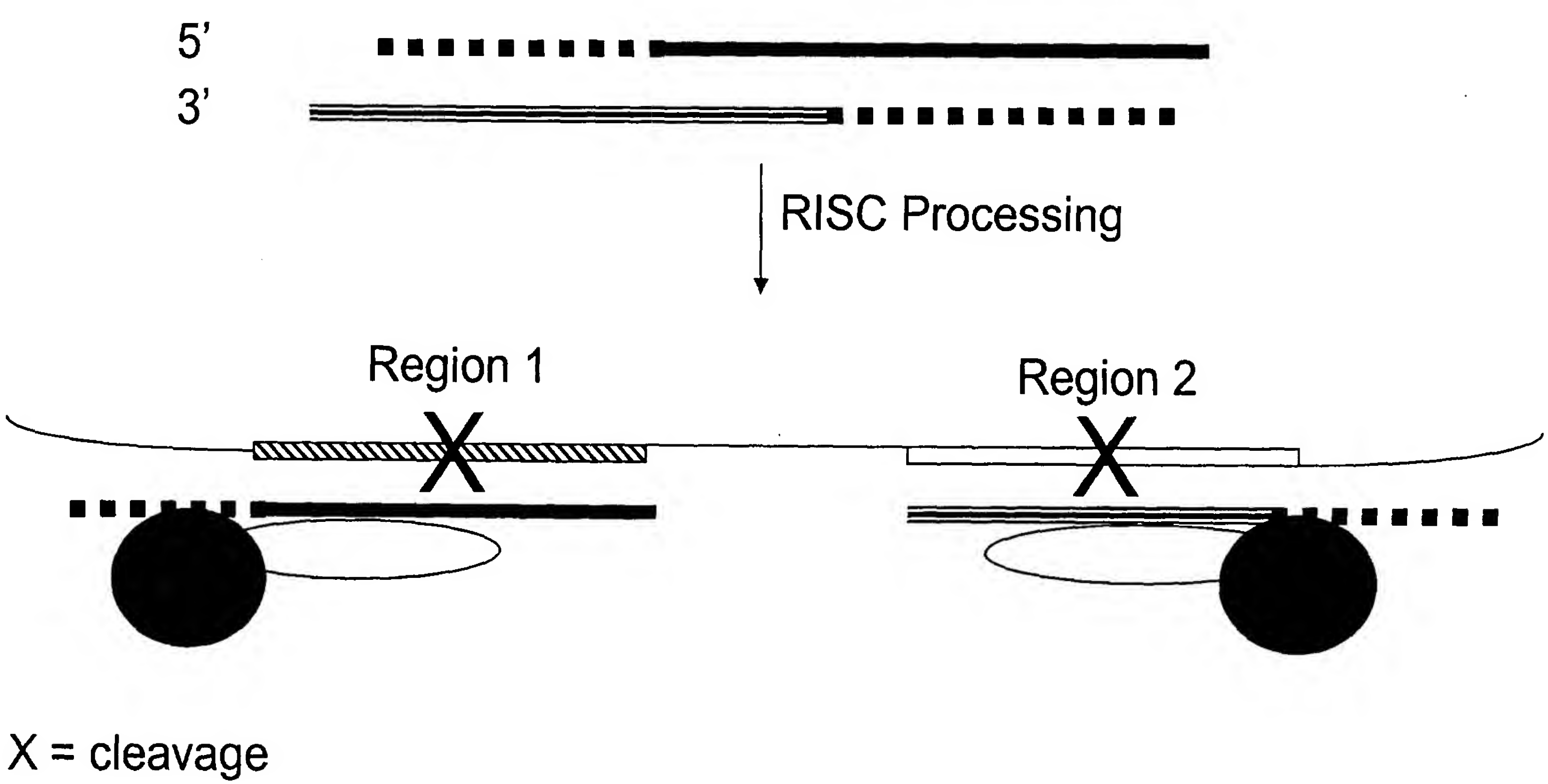


Figure 22 : siNAs targeting HCV chimera

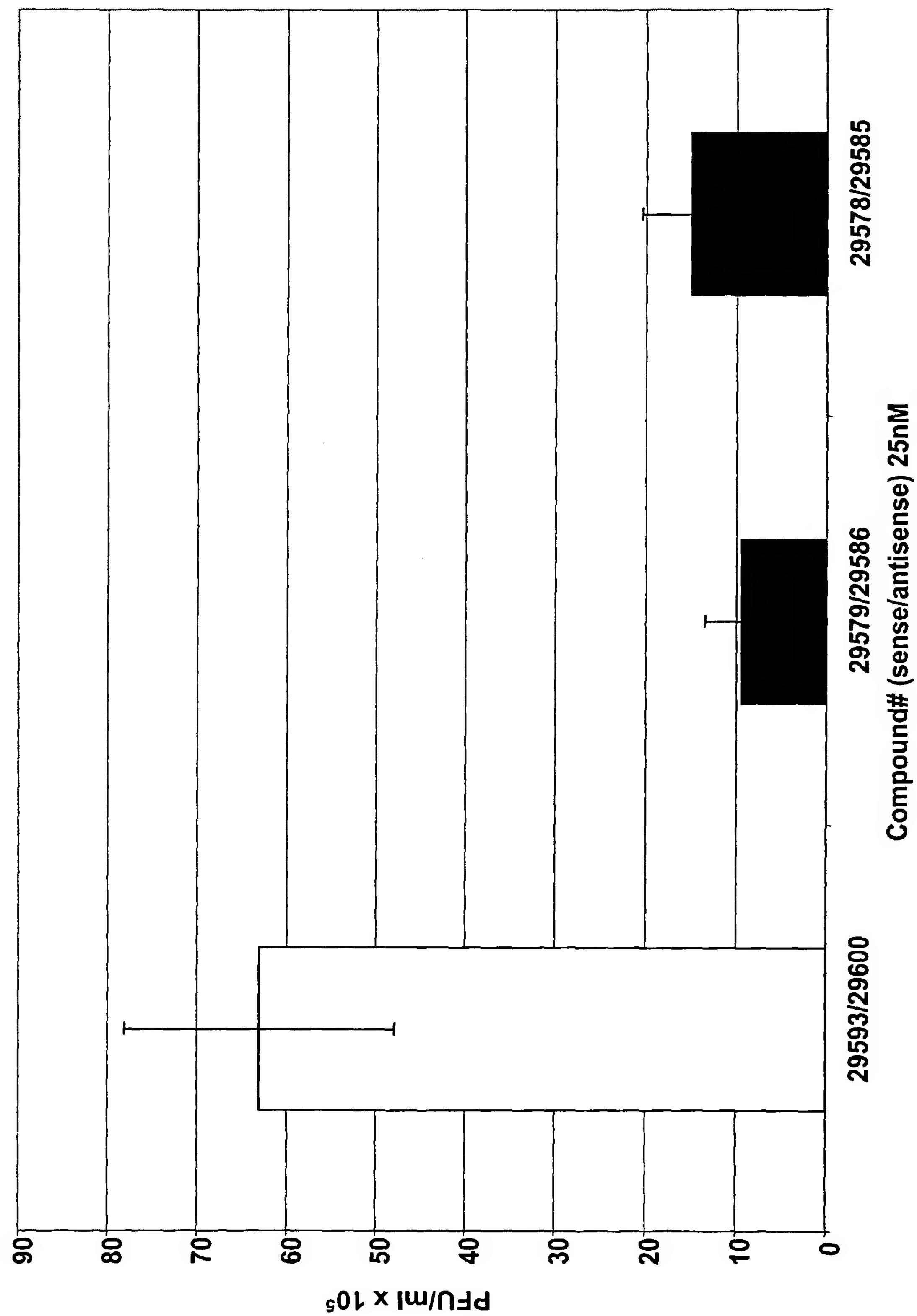


Figure 23: HCV siNA dose response

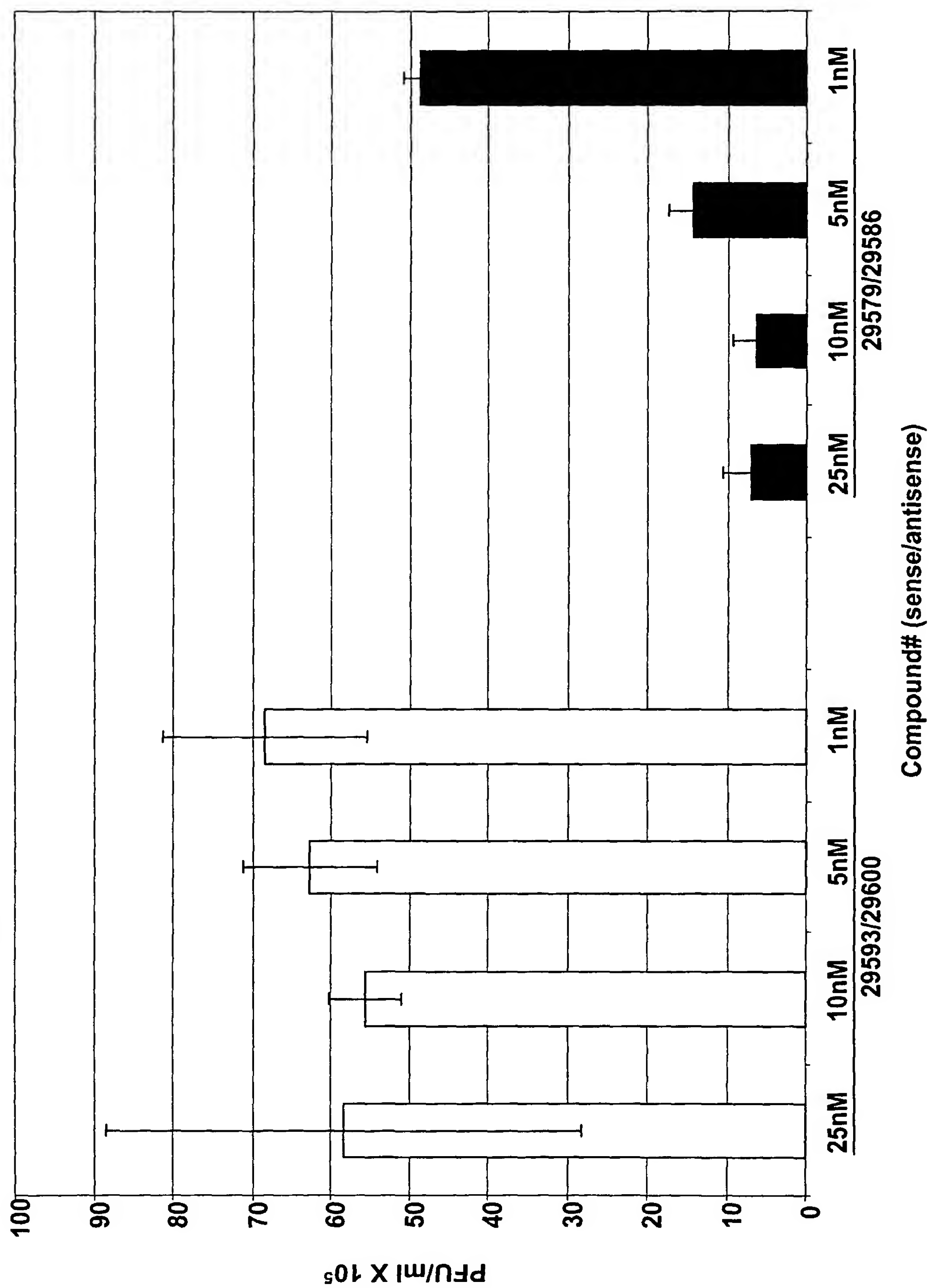


Figure 24: Chemically Modified siNA targeting HCV chimera

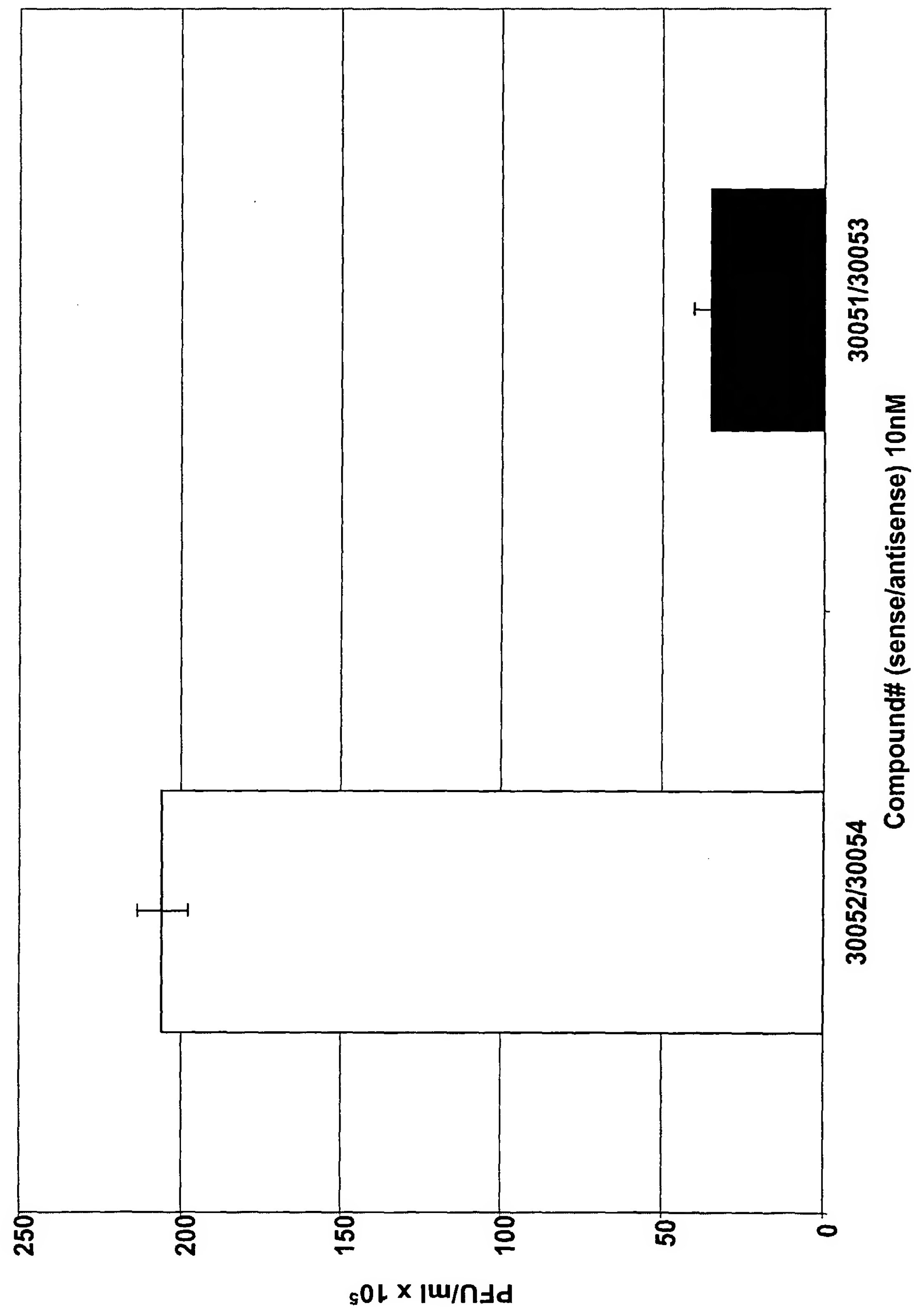


Figure 25: Chemically Modified siNA targeting HCV chimera

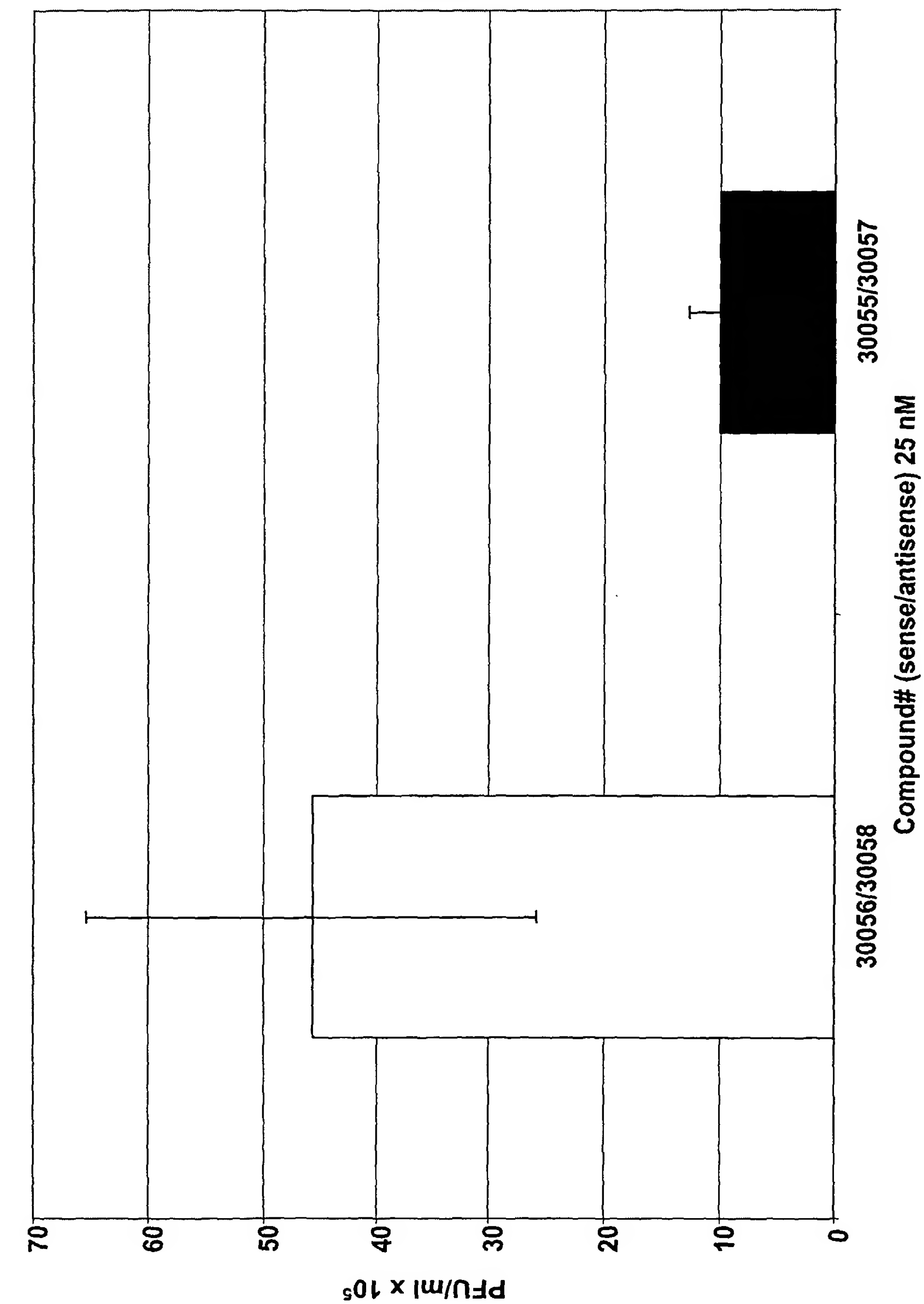


Figure 26: Chemically Modified siNA targeting HCV chimera

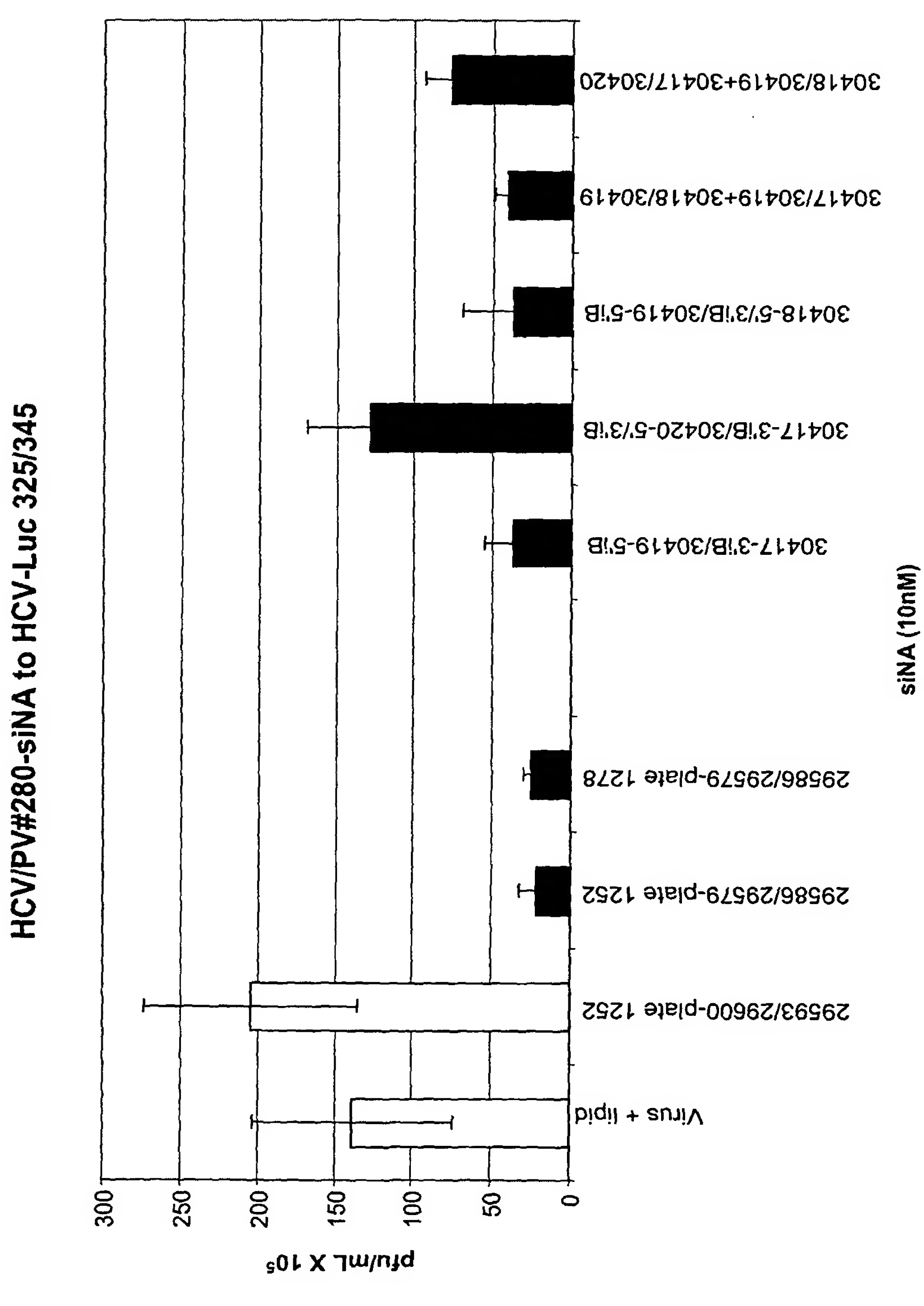


Figure 27: Chemically Modified siNA targeting HCV chimera

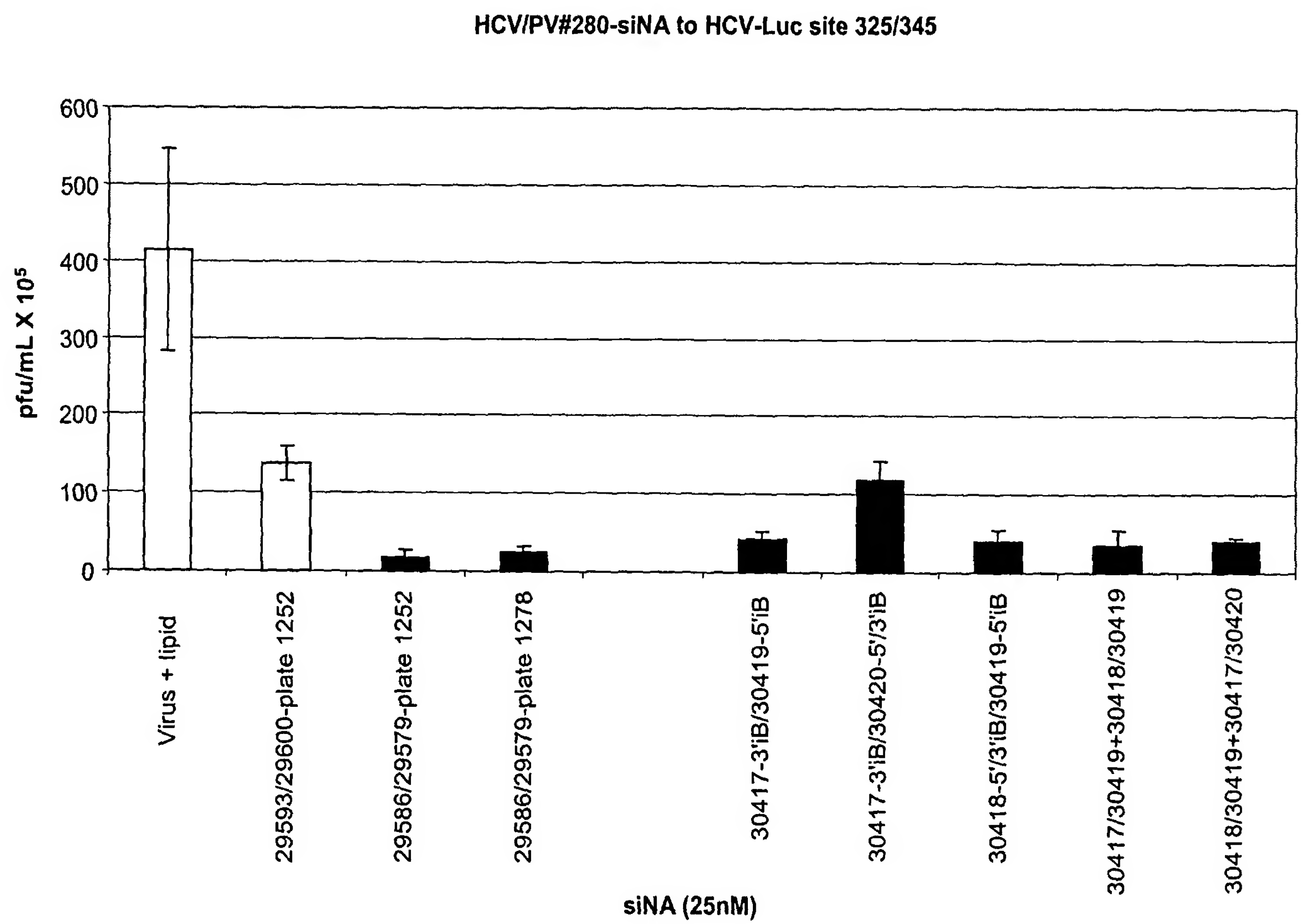


Figure 28: HCV/Replicon Cells transfected with 0.5µl/well LFA 2K-72 hours

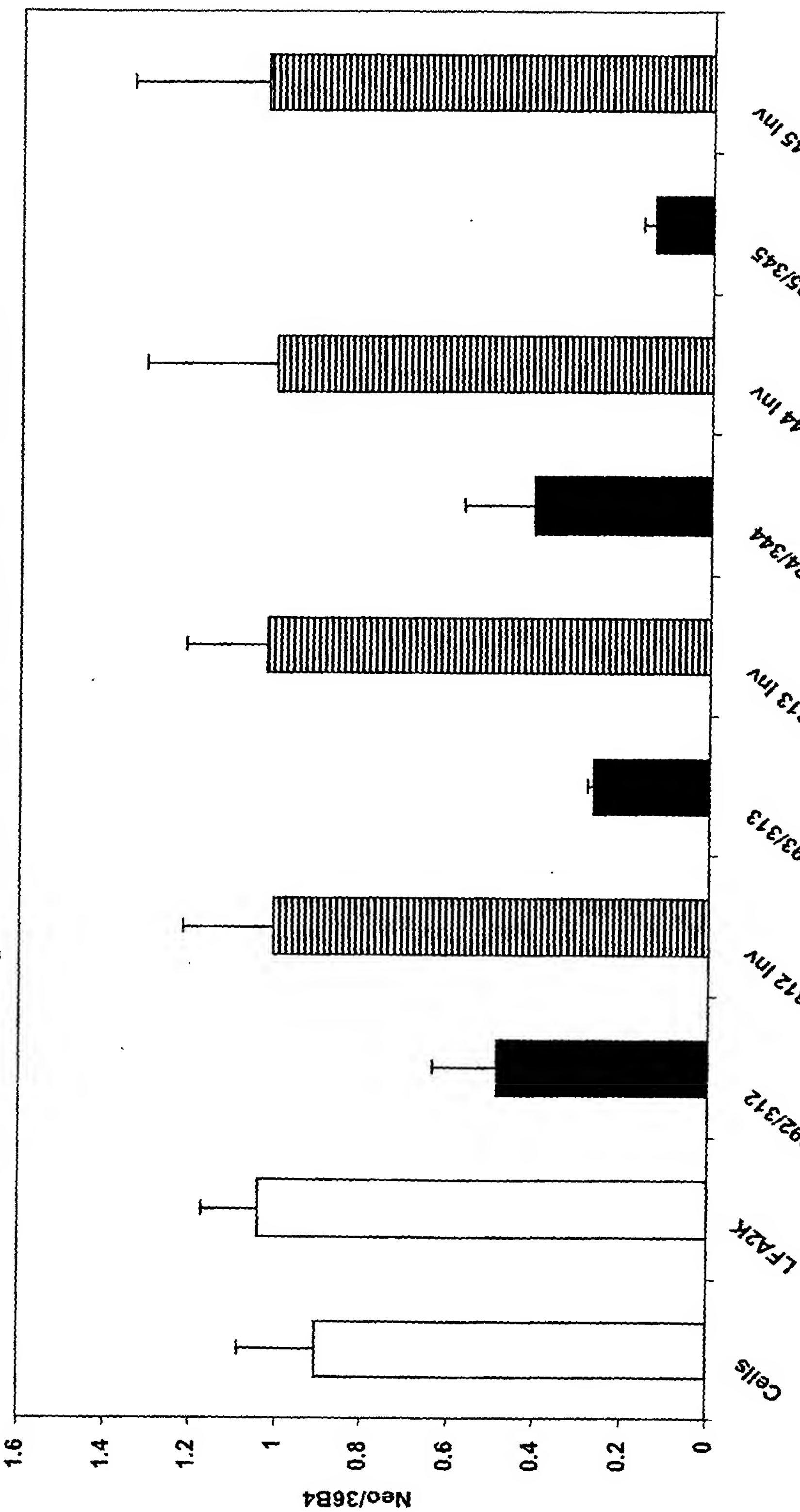


Figure 29: Dose Response with Stab4/5 siNA Leads in HCV Subgenomic Replicon

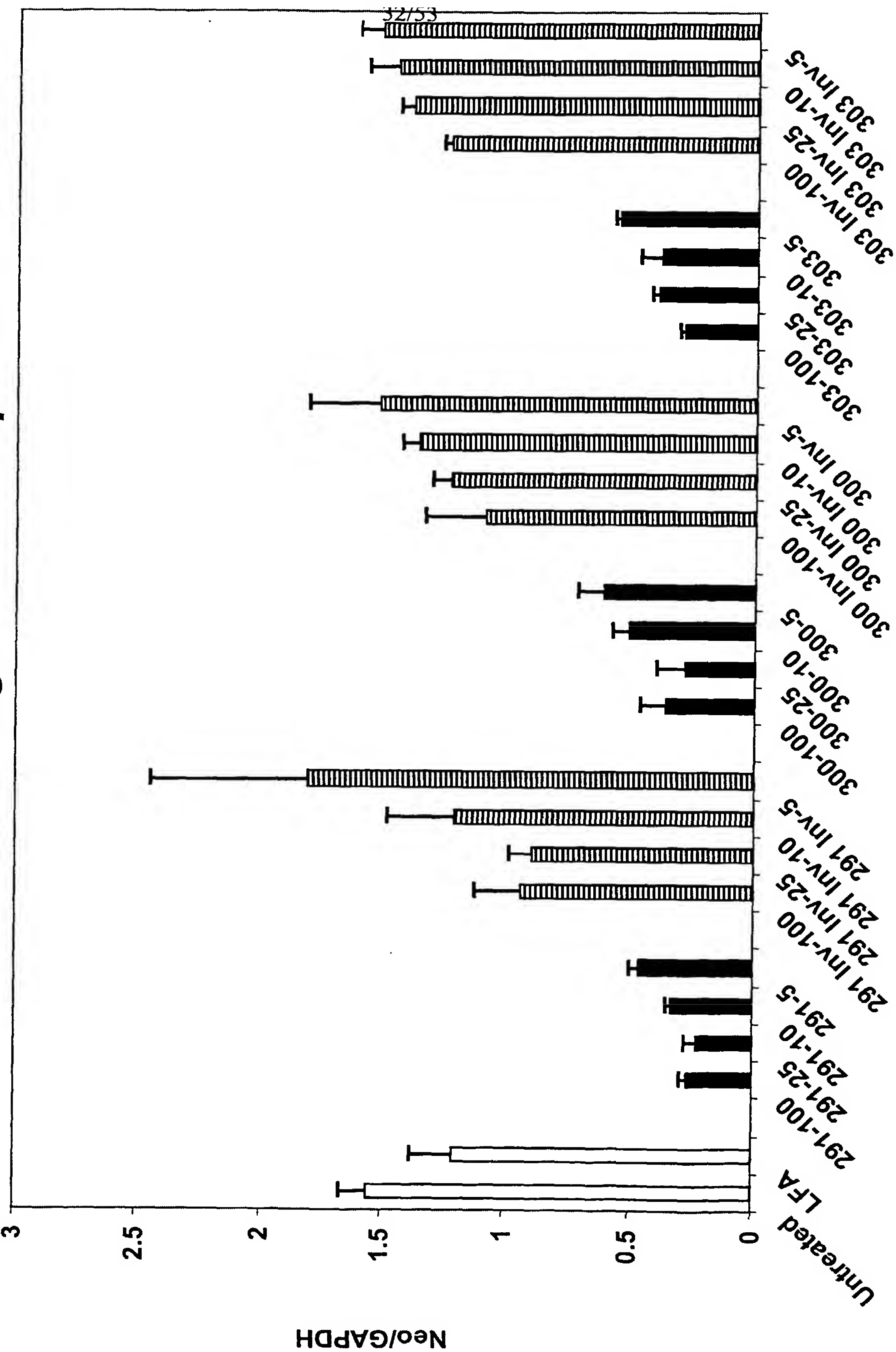


Figure 30: Activity of Stab 7/8 siNA Leads in HCV Subgenomic Replicon

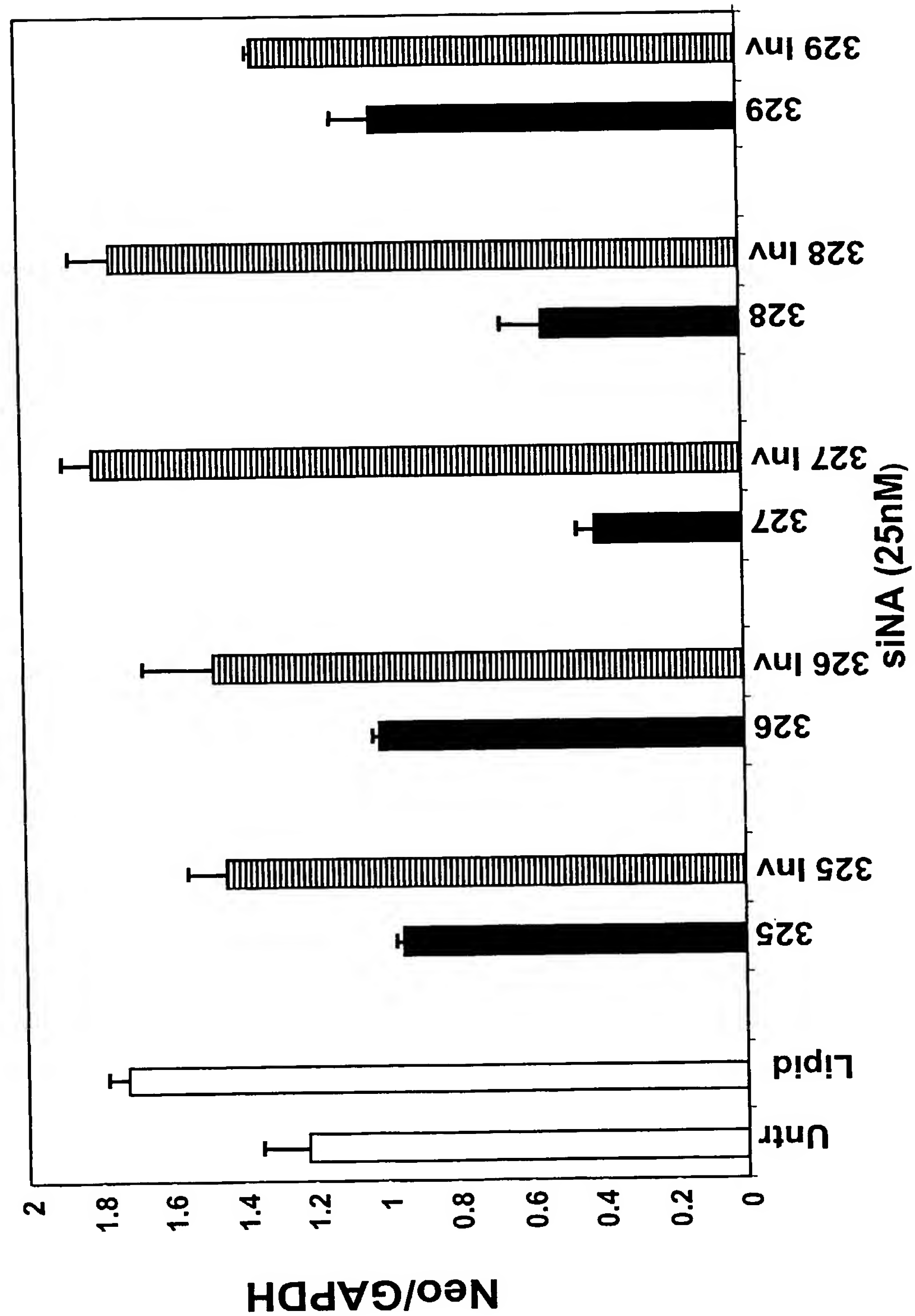


Figure 31: Dose Response with Fully Modified HCV Site 327 siNA

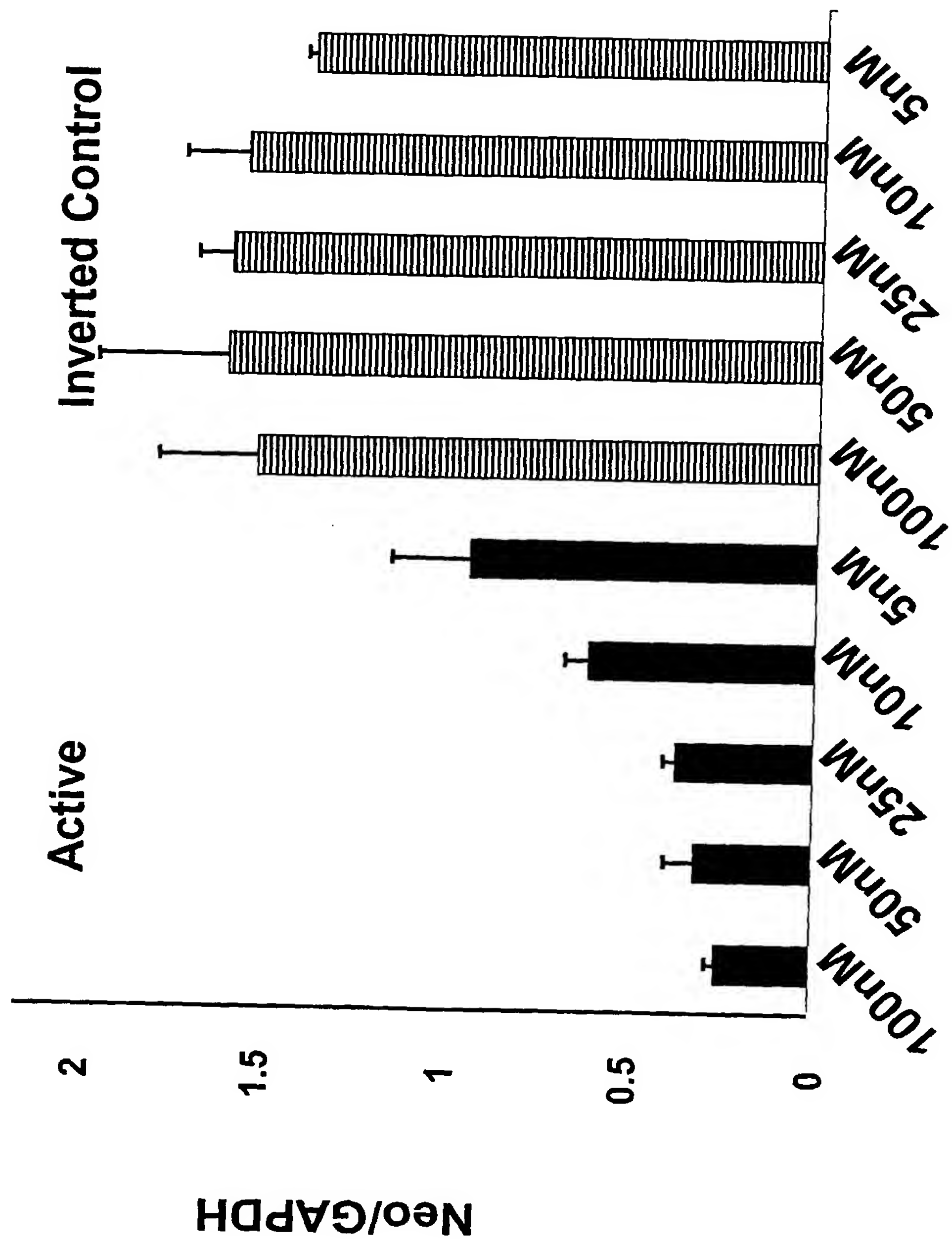


Figure 32: Activity of siNA/Interferon Combination Treatment in HCV Replicon

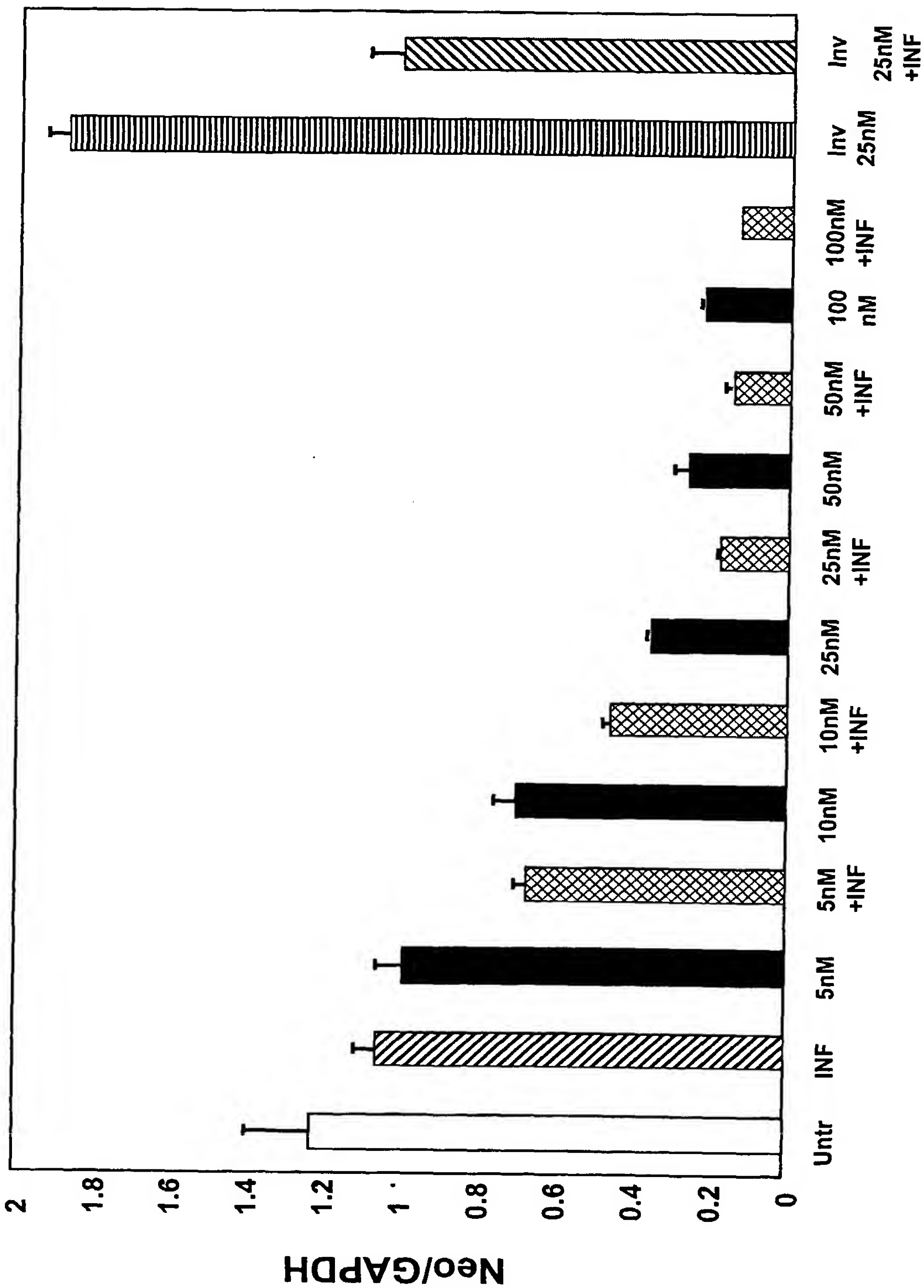


Figure 33: Pooled vs. Multifunctional siNA in HCV Replicon System

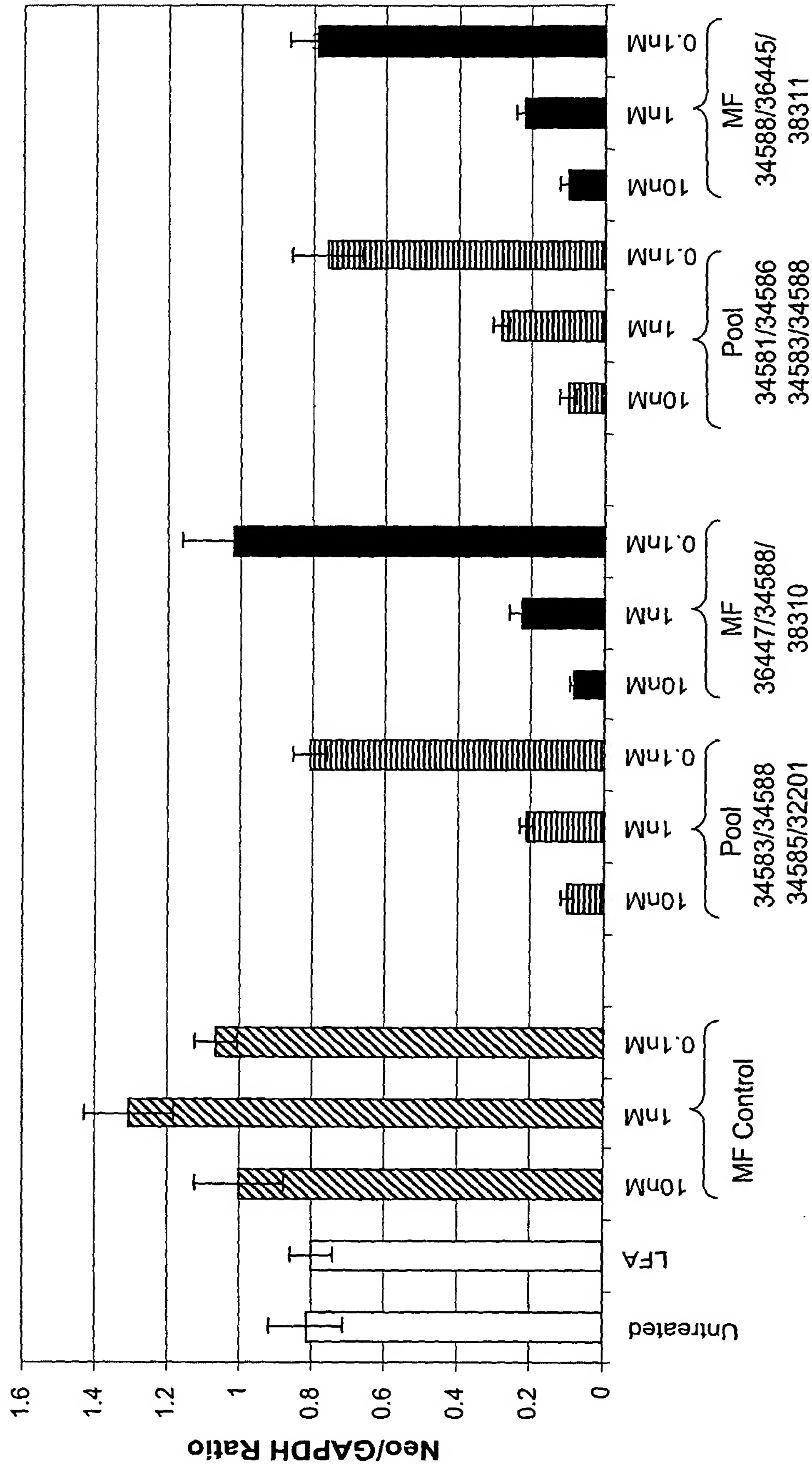


Figure 34: Pooled vs. Multifunctional siNA in HCV Replicon System

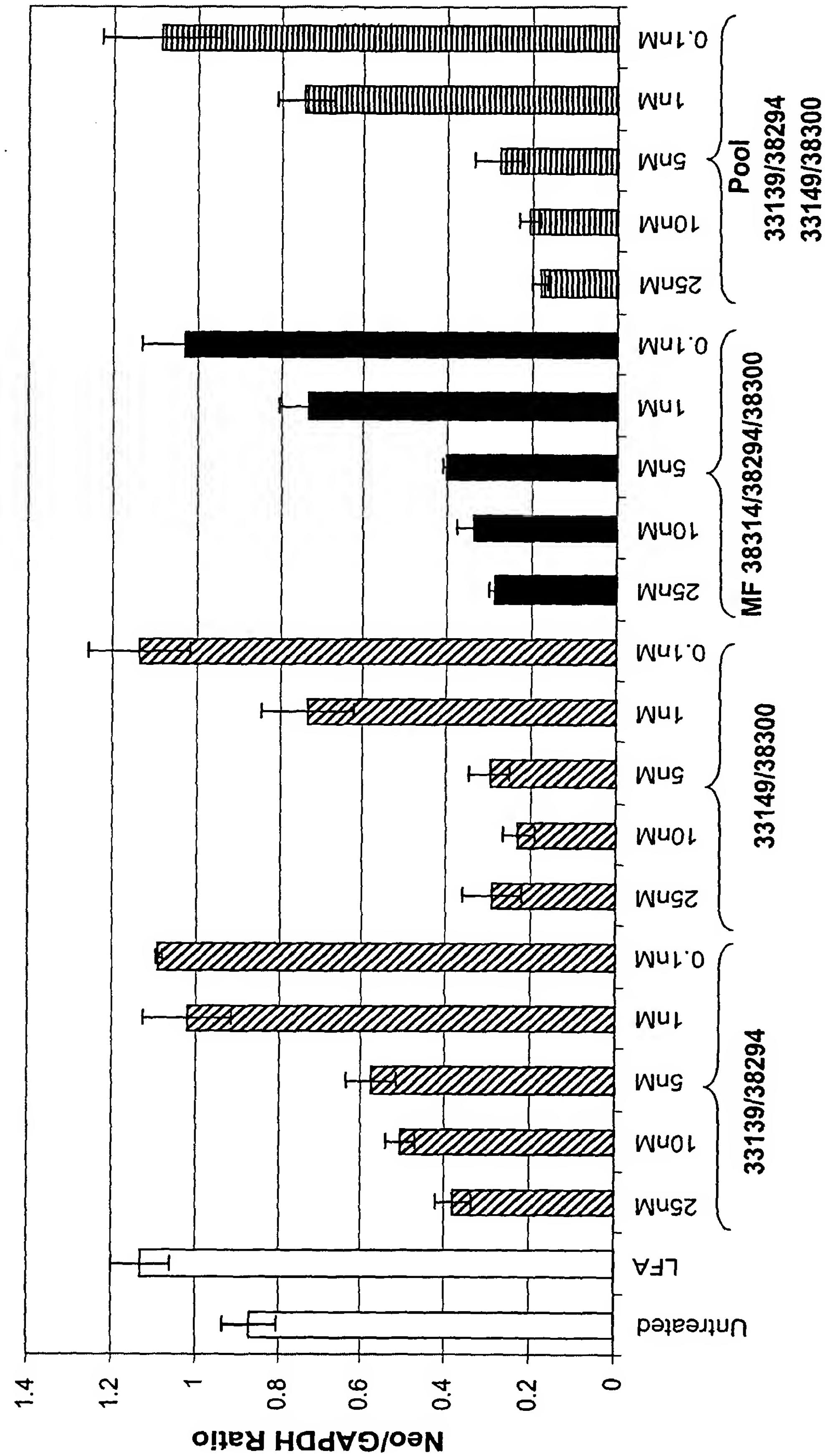


Figure 35: Pooled vs. Multifunctional siNA in HCV Replicon System

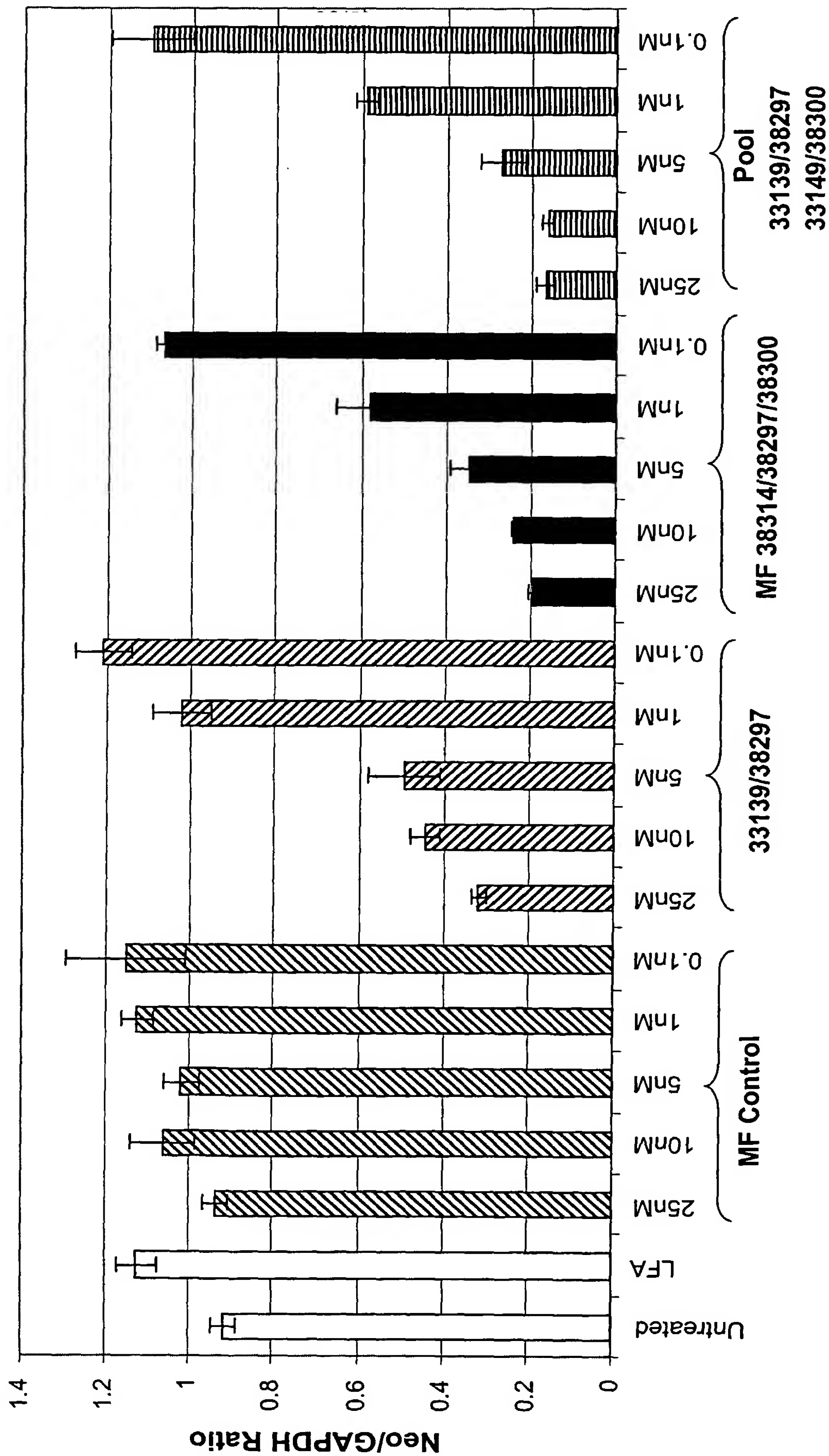


Figure 36: Pooled vs. Multifunctional siNA in HCV Replicon System

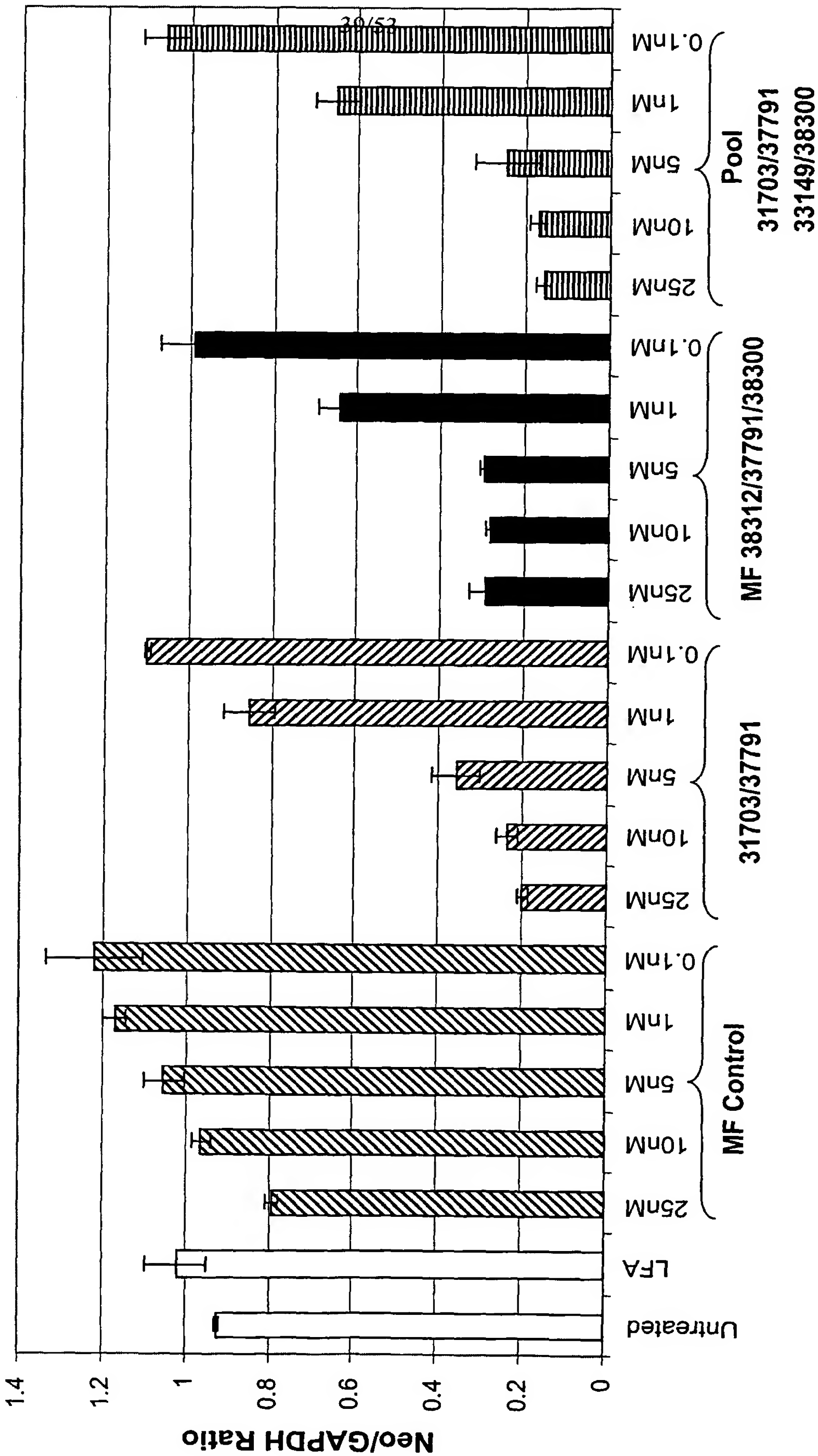


Figure 37: Pooled vs. Multifunctional siNA in HCV Replicon System

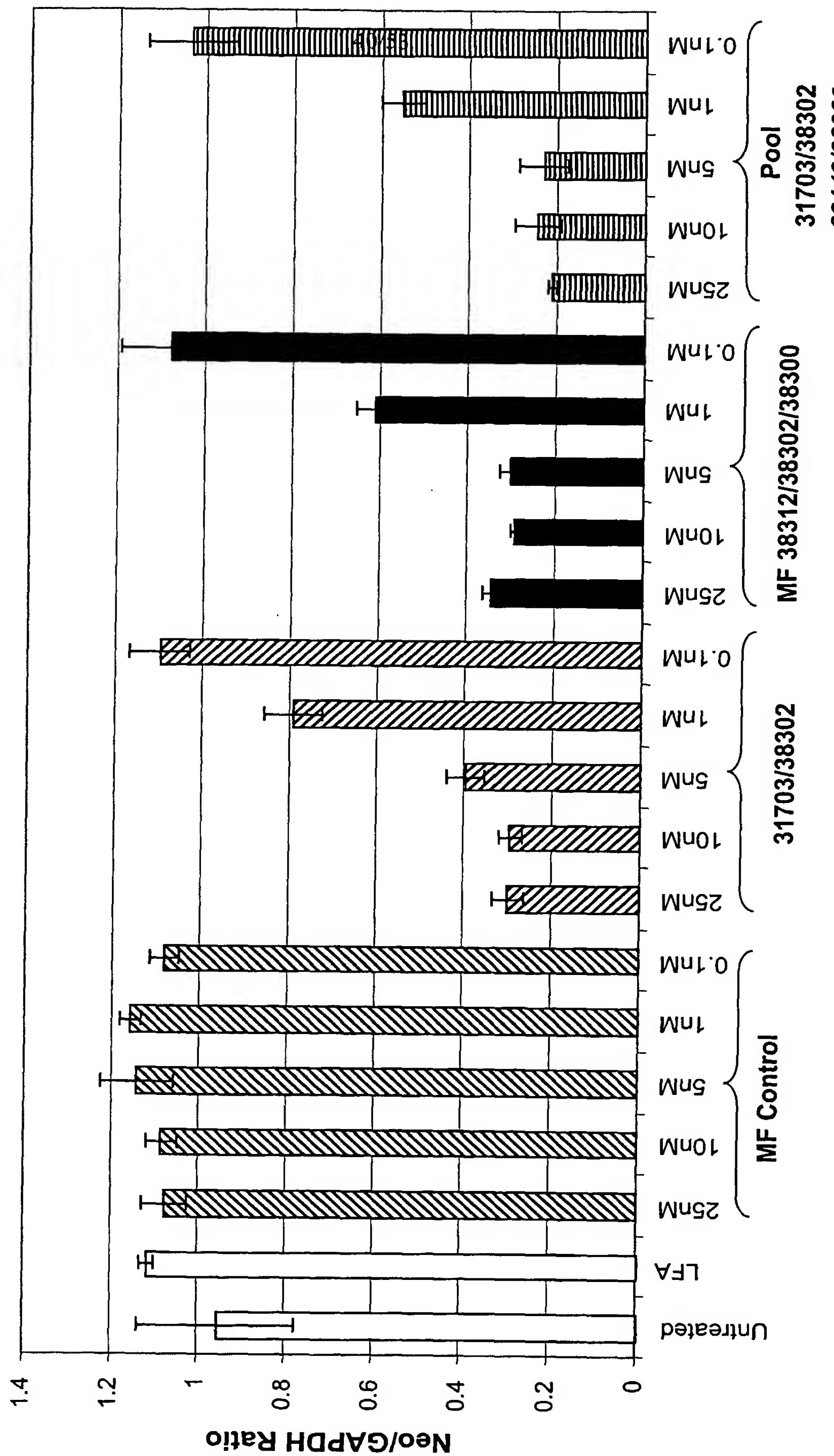


Figure 38: Pooled vs. Multifunctional siNA in HCV Replicon System

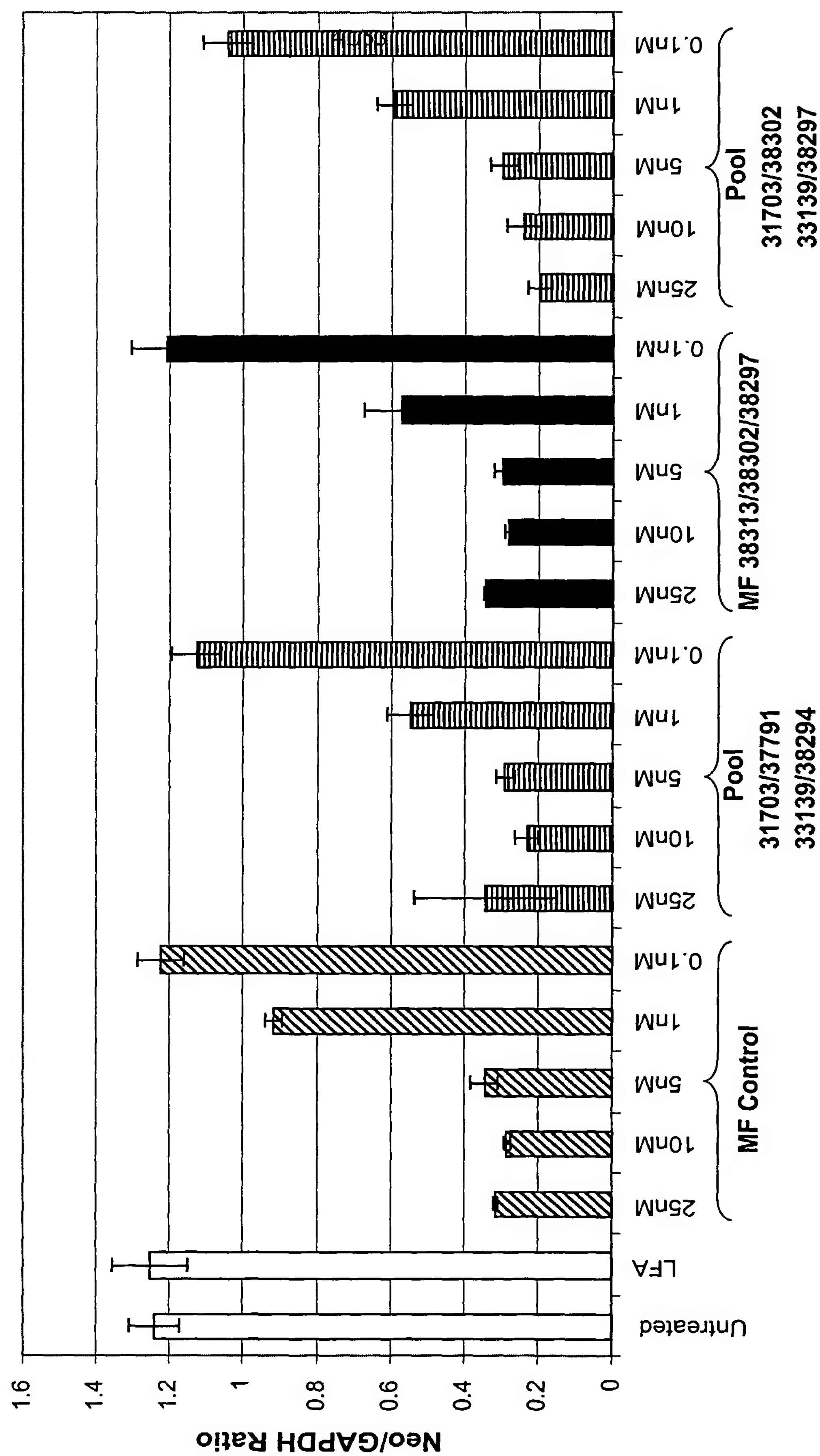


Figure 39: Pooled vs. Multifunctional siNA in HCV Replicon System

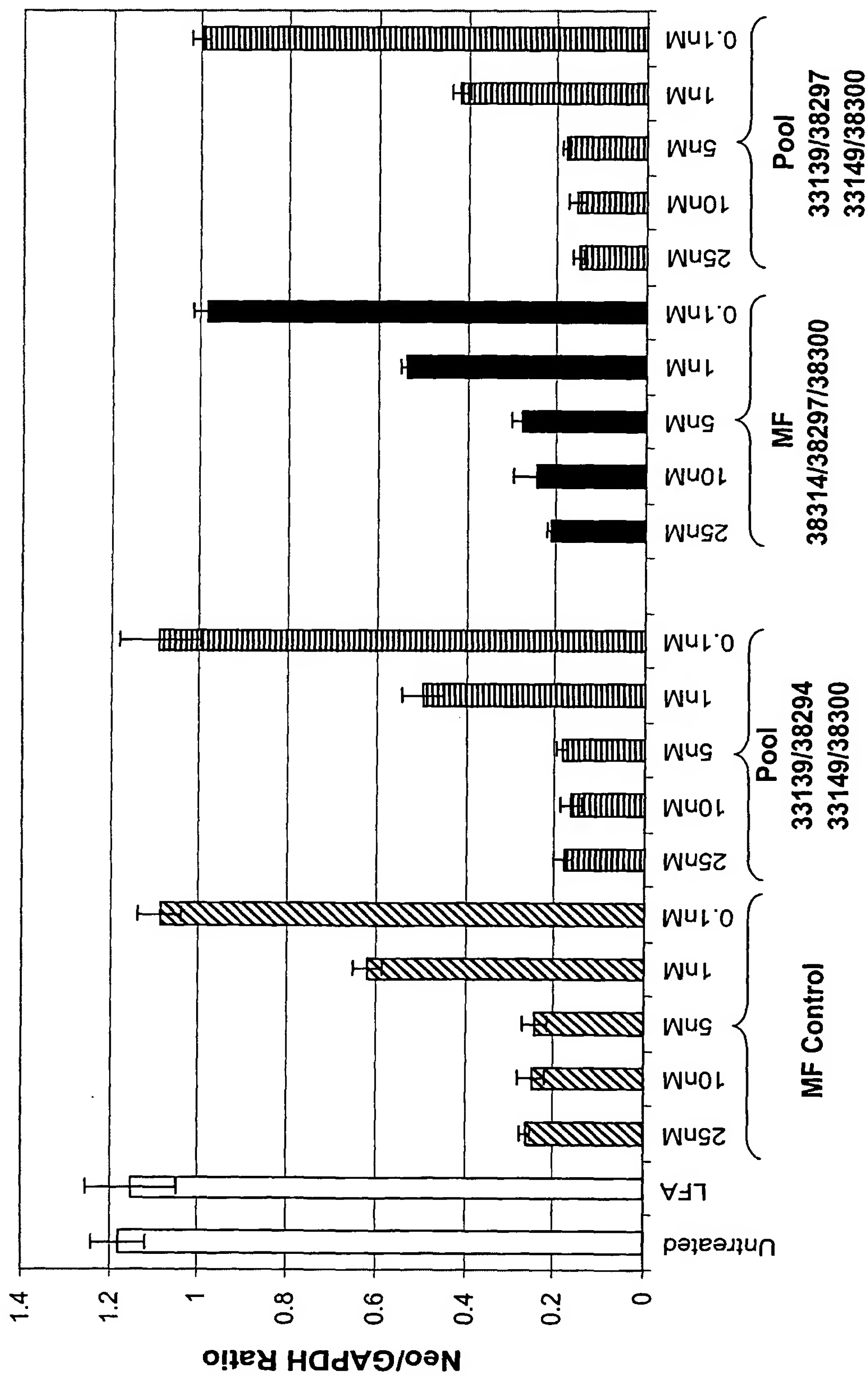


Figure 40: Pooled vs. Multifunctional siNA in HCV Replicon System

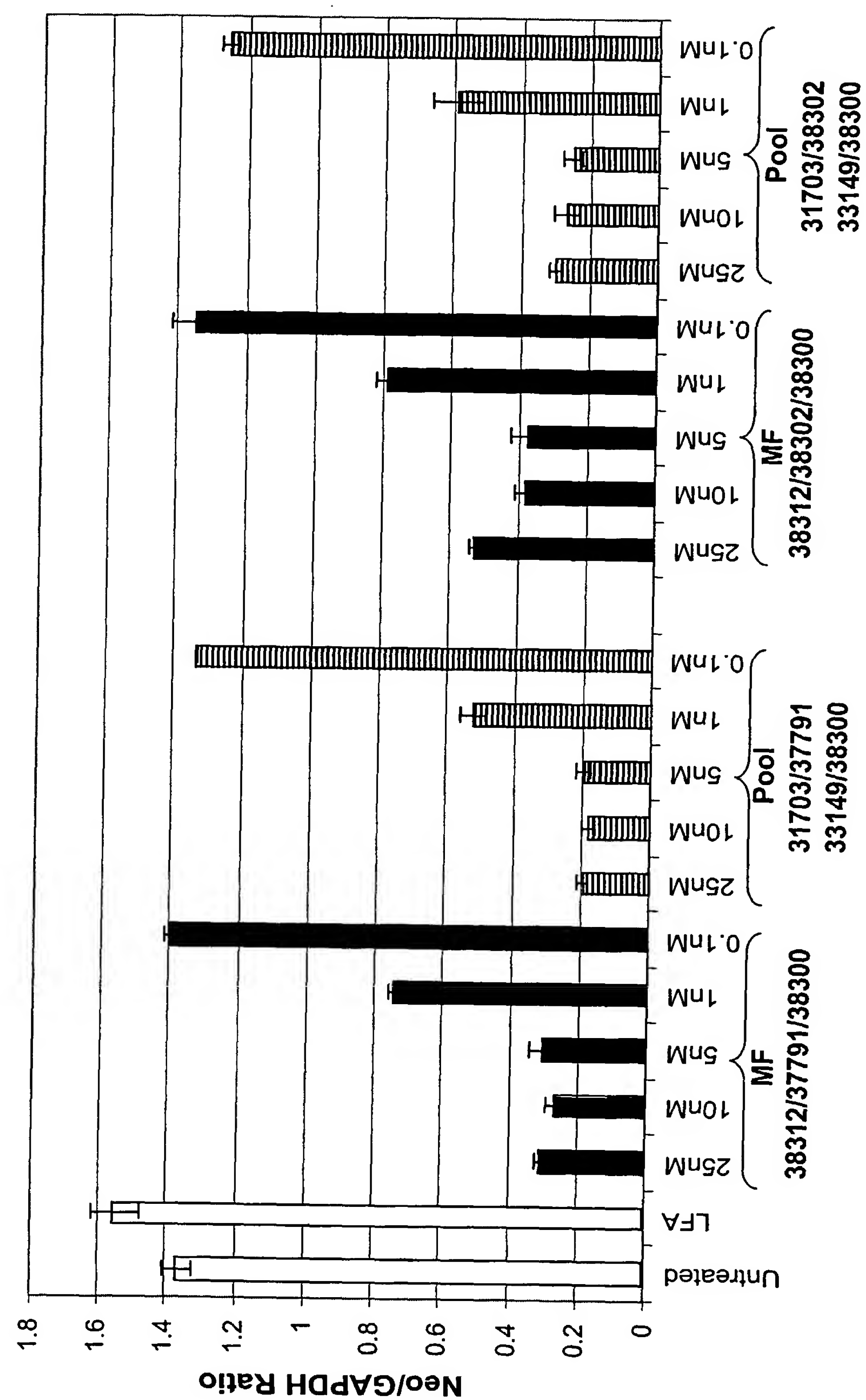


Figure 41: Pooled vs. Multifunctional siNA in HCV Replicon System

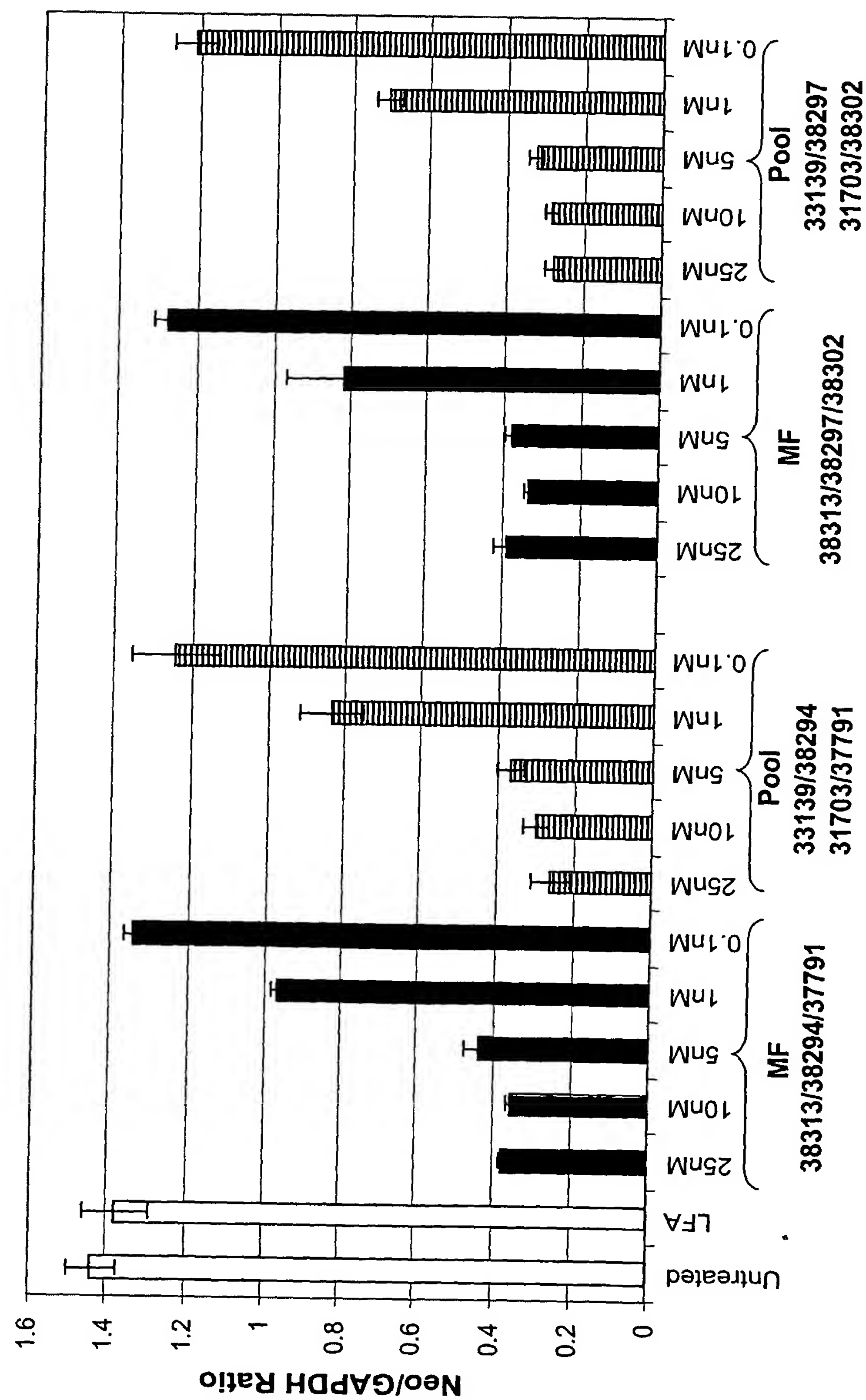
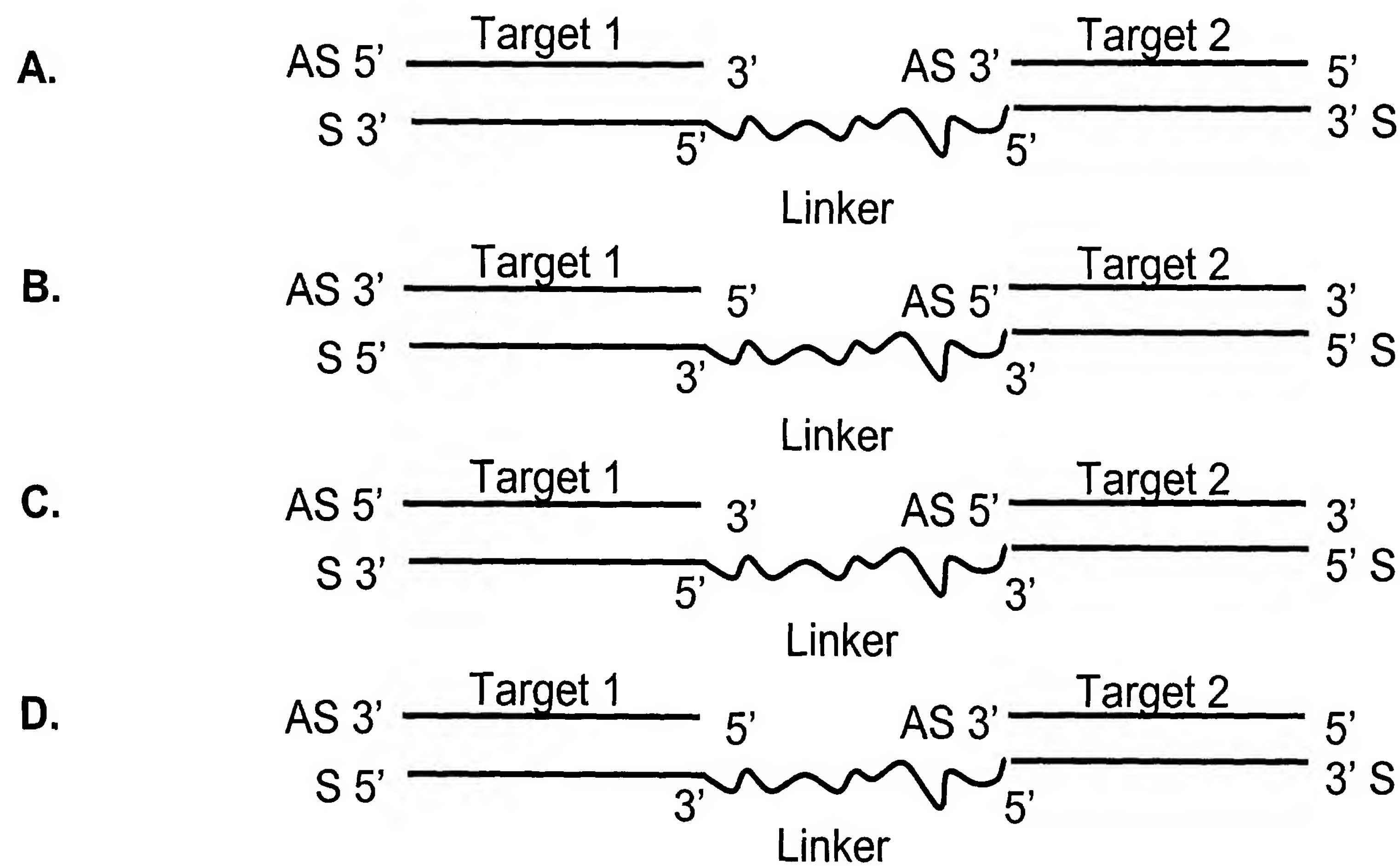
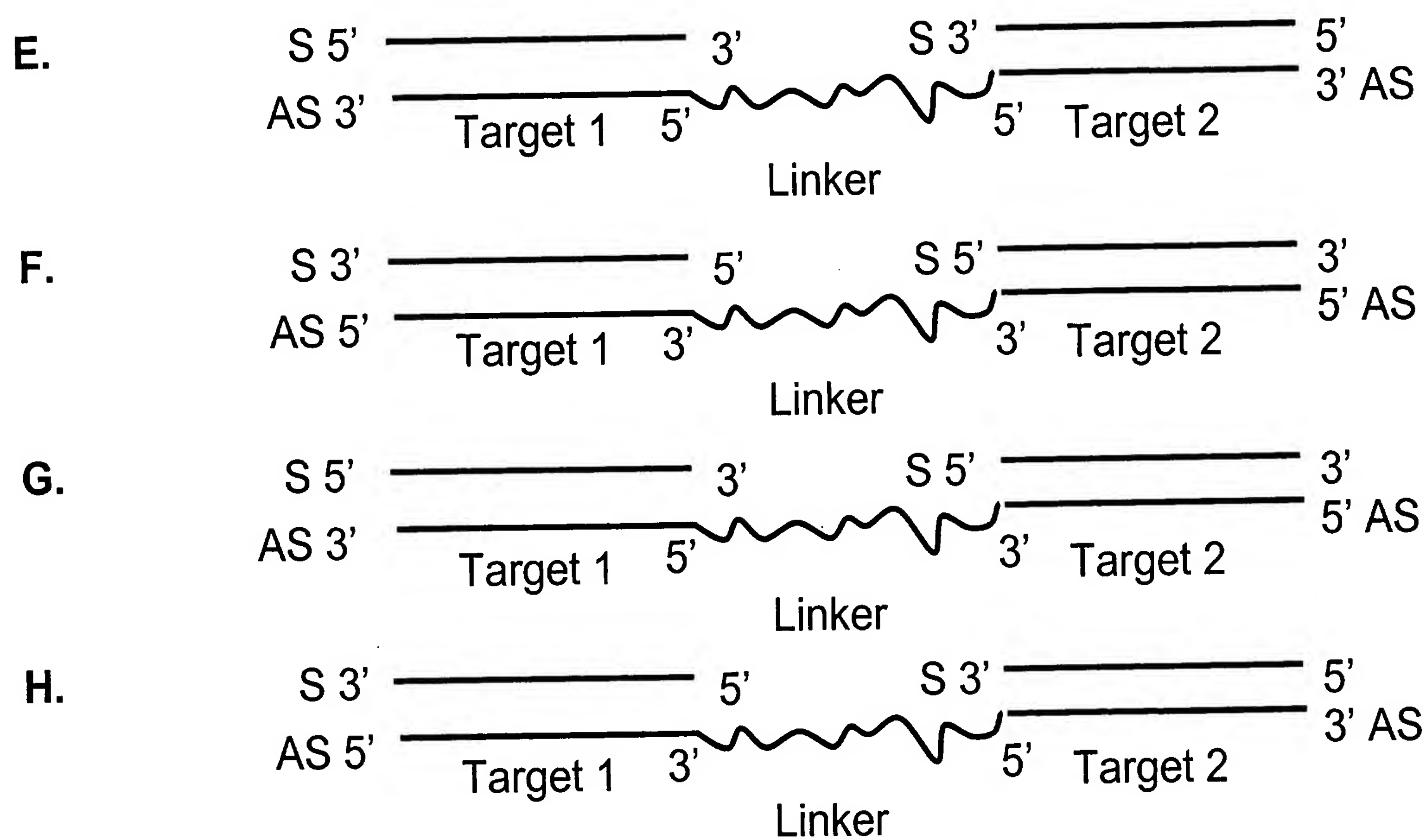


Figure 42: Tethered Multifunctional siNA design



S = sense, AS = antisense
Linker region can be nucleotide or non-nucleotide linker, and can optionally be decorated, for example with conjugates polymers or aptamers, such as for delivery purposes.

Figure 42: Tethered Multifunctional siNA design



S = sense, AS = antisense
Linker region can be nucleotide or non-nucleotide linker, and can optionally be decorated, for example with conjugates polymers or aptamers, such as for delivery purposes.

Figure 43: Dendrimer Multifunctional siNA designs

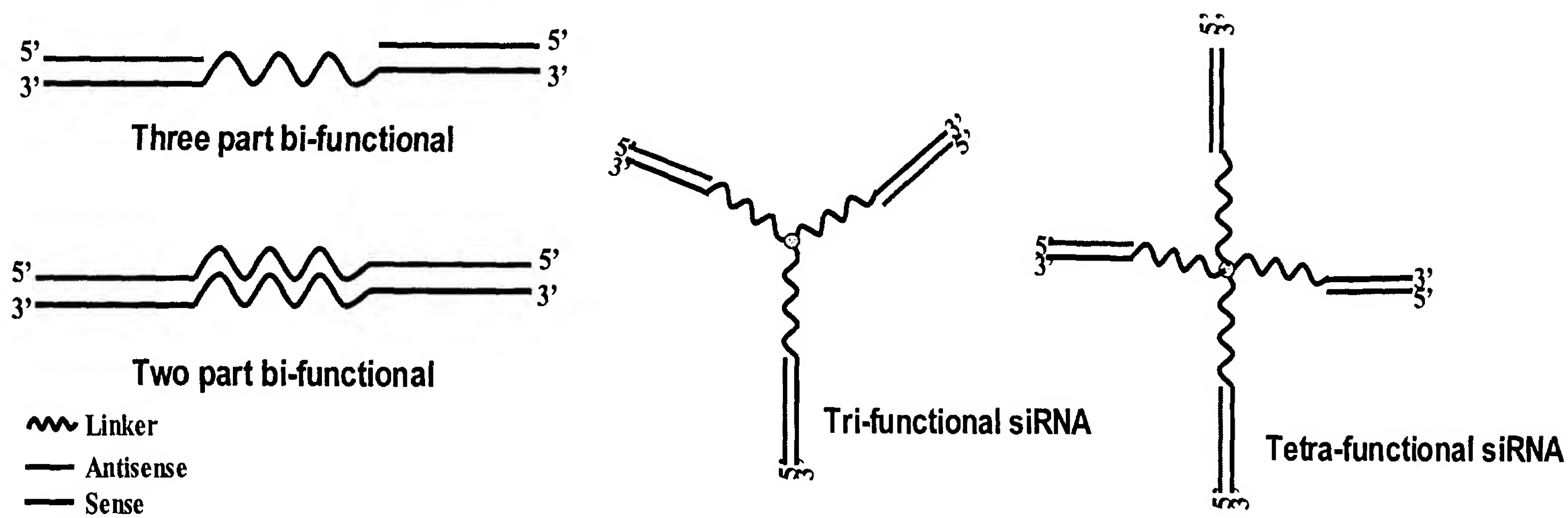


Figure 44: Supramolecular Multifunctional siNA designs

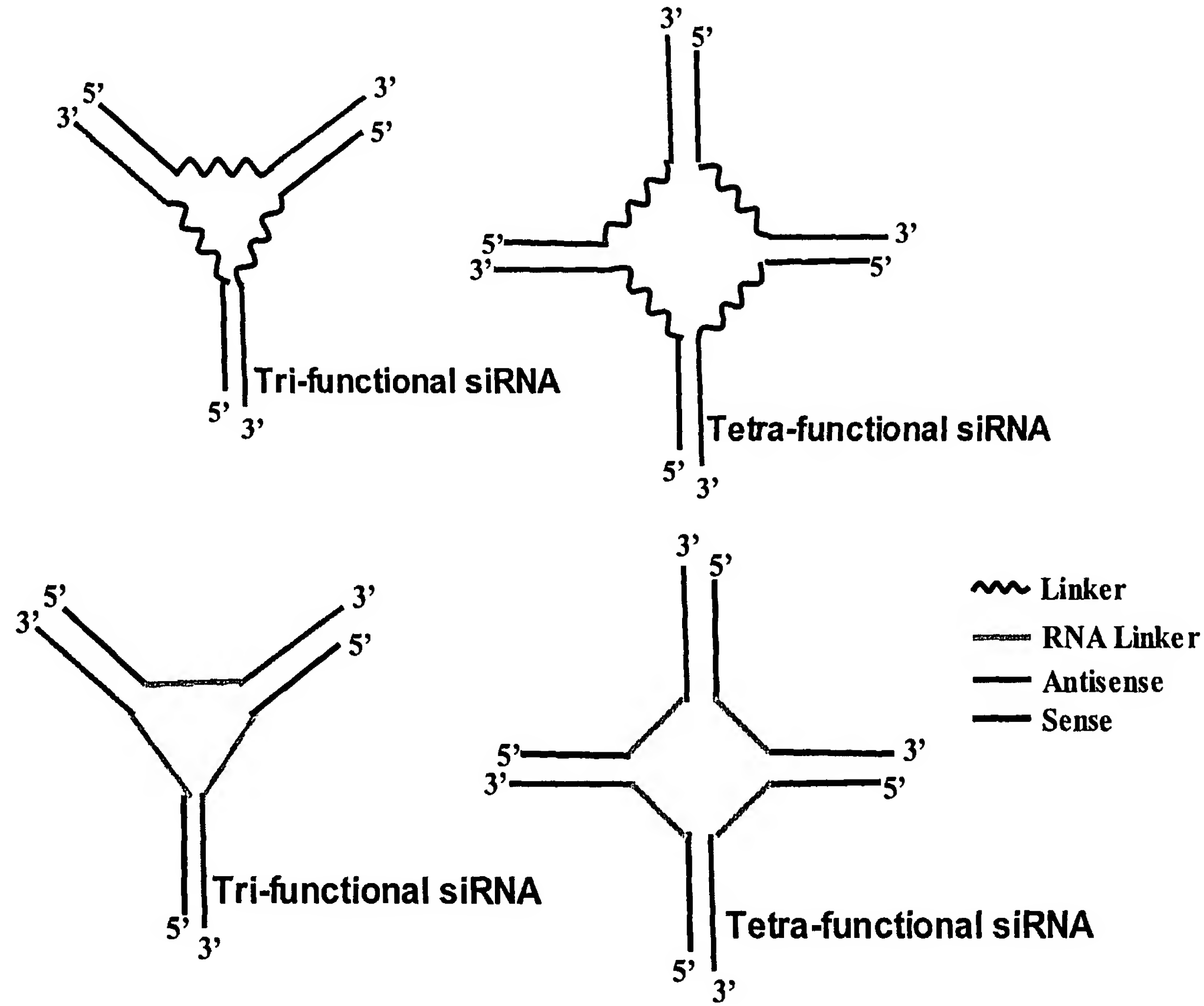


Figure 45: Dicer enabled multifunctional siNA design

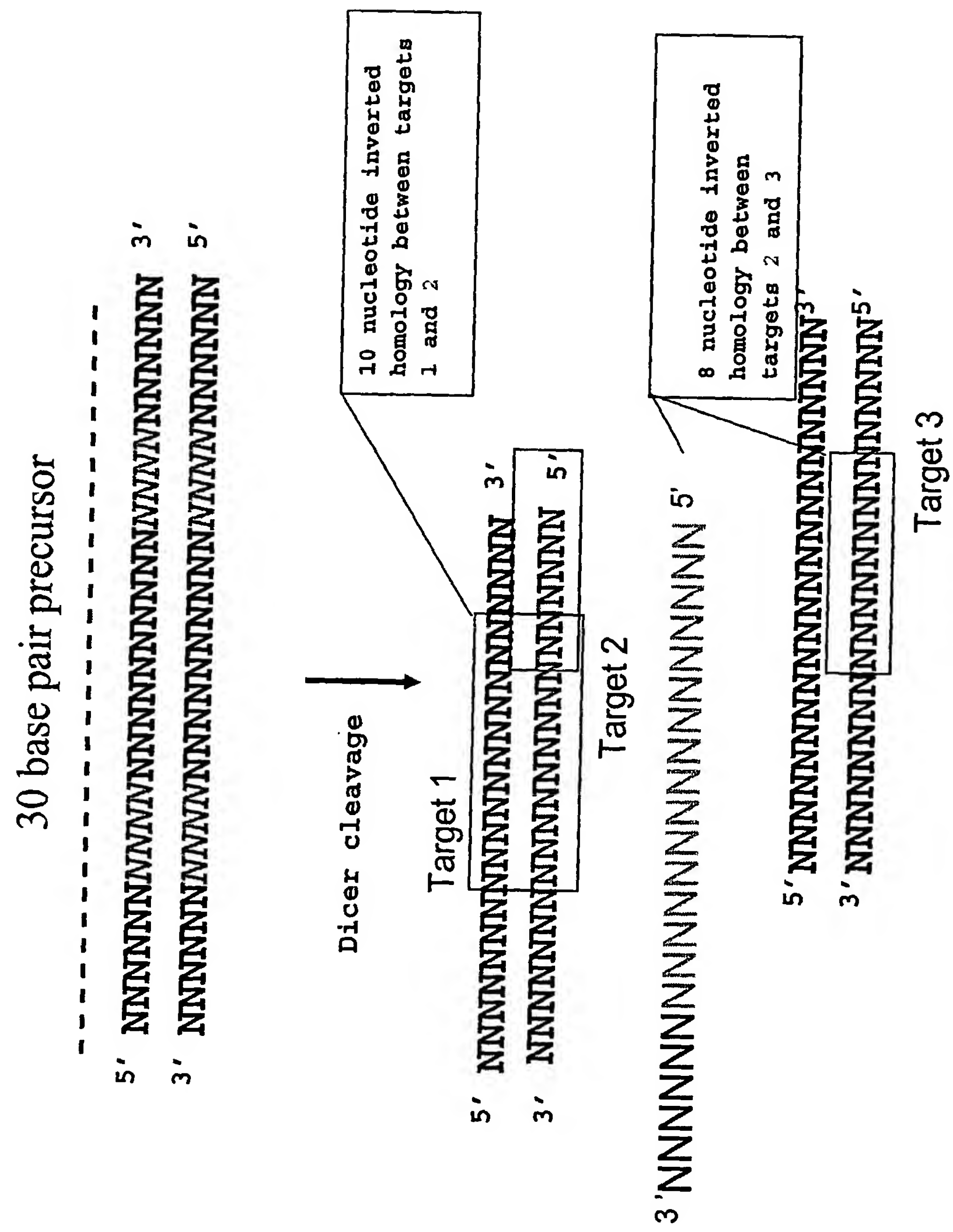


Figure 46: Dicer enabled multifunctional siNA design

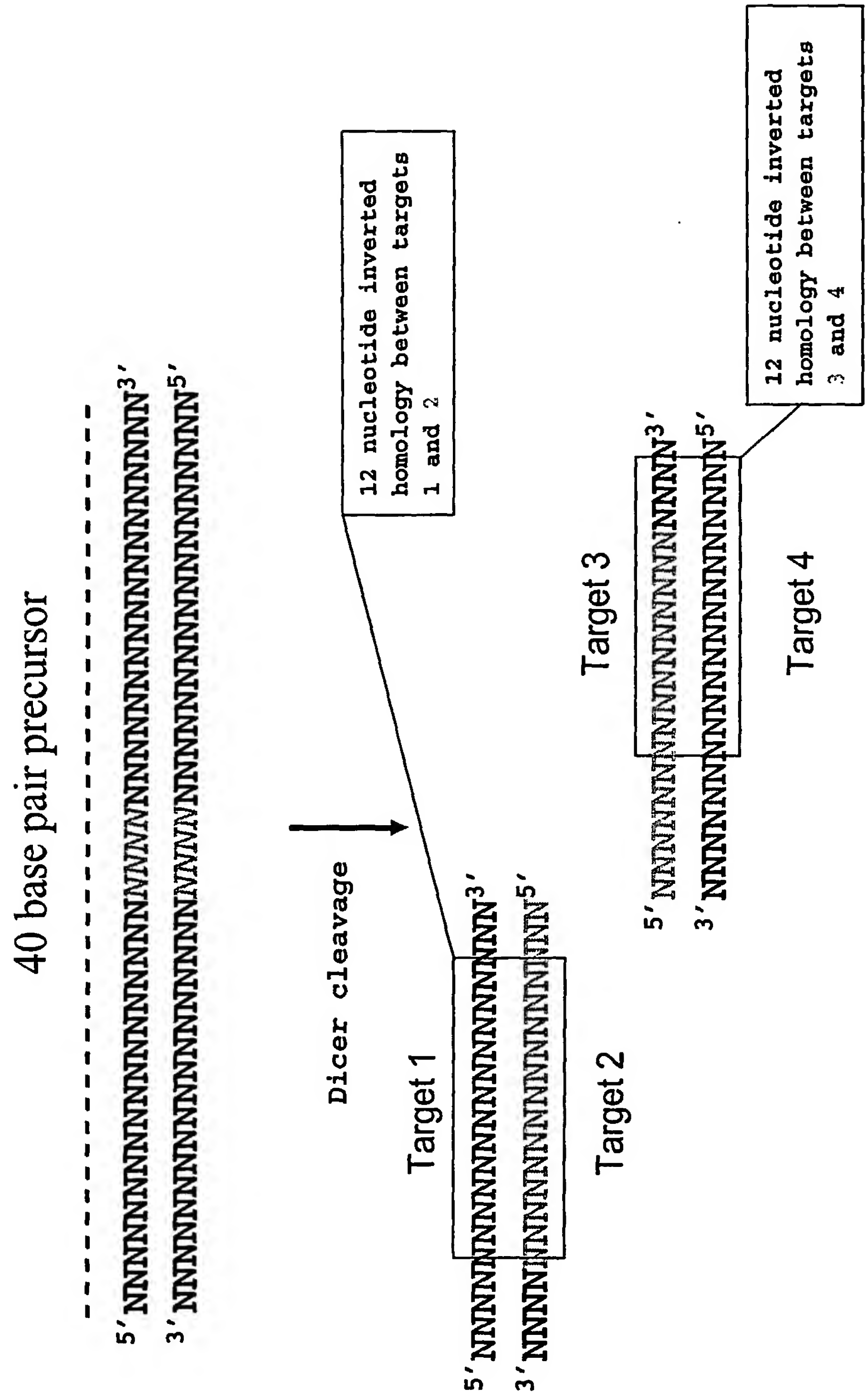


Figure 47: siNA base pair walk

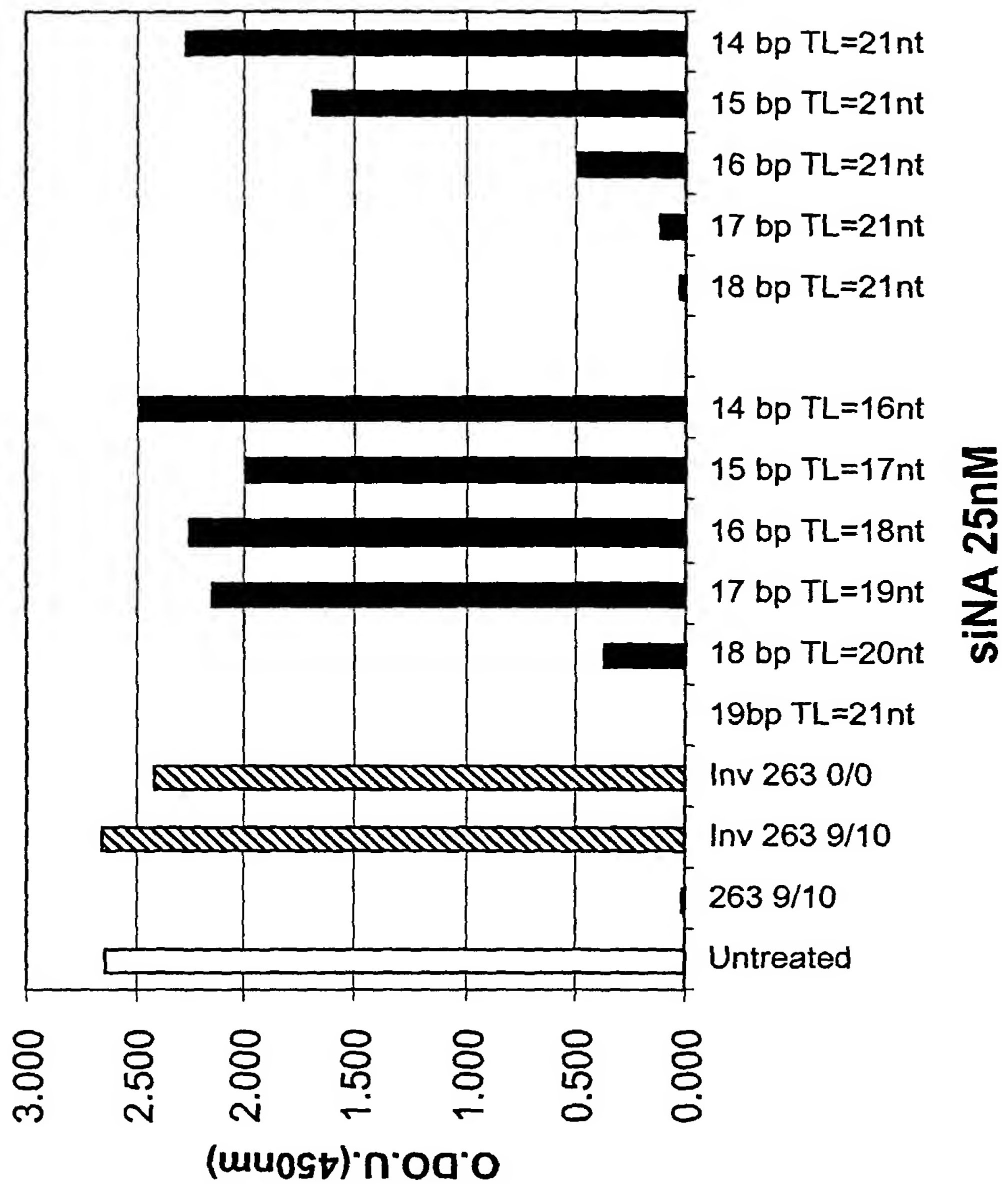


Figure 48: Additional Multifunctional siNA designs

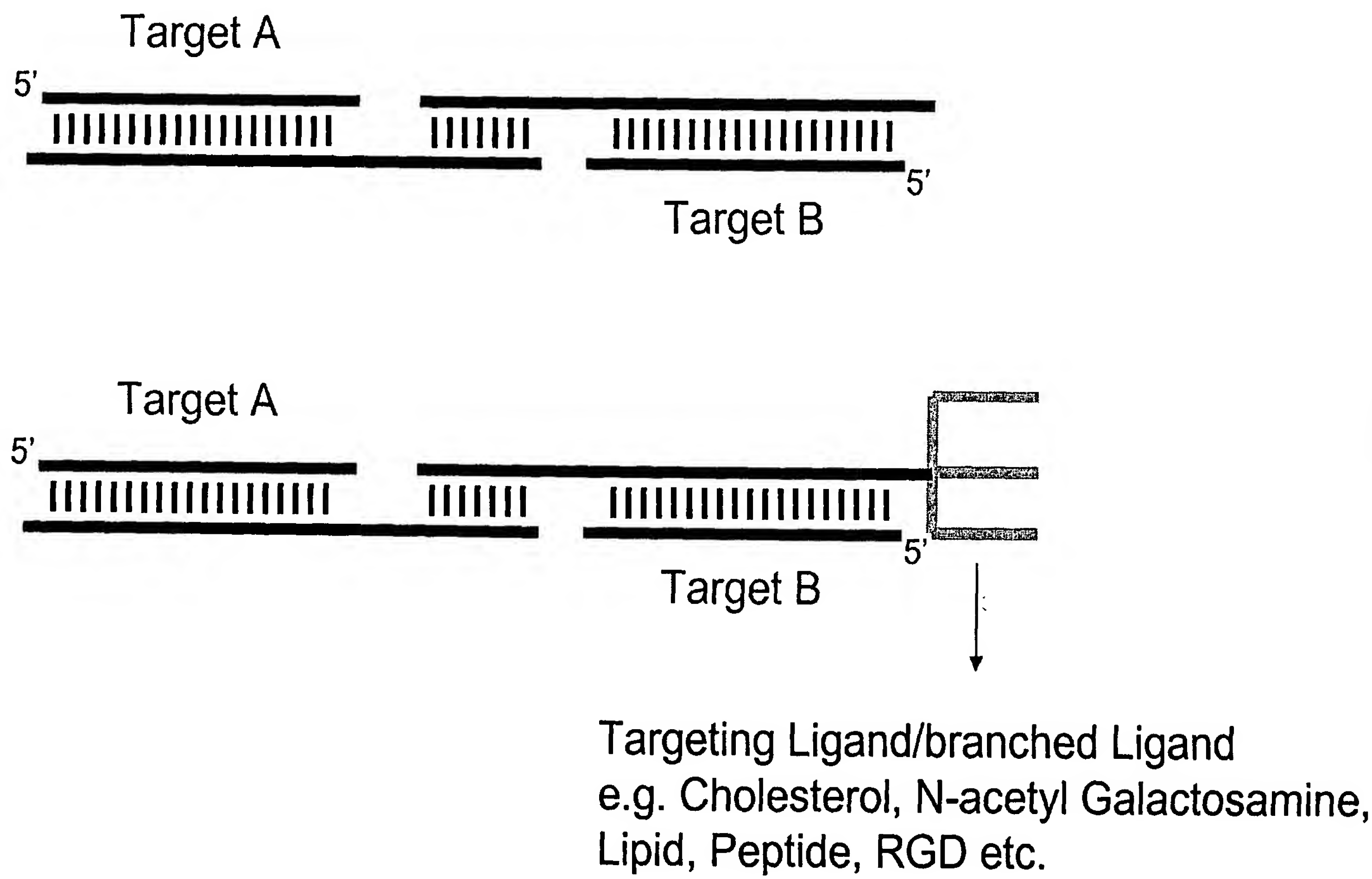


Figure 49: Additional Multifunctional siNA designs